Bayer CropScience Petition (08-340-01p) FOR Determination of Nonregulated Status of Insect Resistant and Glufosinate Ammonium-Tolerant (TwinLink™) Cotton, *Gossypium hirsutum*, Events T304-40 x GHB119

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ACRONYMS

AIA	advanced informed agreement
AMS	Agricultural Marketing Service
ANZFA	Australia and New Zealand Food Authority
AOSCA	Association of Official Seed Certifying Agencies
APHIS	Animal and Plant Health Inspection Service
ARMS	Agricultural Resource Management Survey
BCS	Bayer Crop Science
BRAD	Biopesticides Registration Action Document
BRS	Biotechnology Regulatory Service
BXN	bromoxynil-tolerant
CBD	Convention on Biological Diversity
CBW	cotton bollworm
CEQ	Council of Environmental Quality
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations
DNA	deoxyribonucleic acid
EA	Environmental Assessment
ECOS	Environmental Conservation Online System
ELS	extra-long staple
EO	Executive Order
EPA	Environmental Protection Agency
ERA	ecological risk assessment
ESA	Endangered Species Act
EU	European Union
FAW	fall armyworm
FDA	Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FFP	food, feed, or processing
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FR	Federal Register
GE	genetically engineered
GHG	greenhouse gases
IPCC	Intergovernmental Panel on Climate Change
LMO	living modified organism
MFA	Multifibre Arrangement
MOU	memorandum of understanding
N_2O	nitrous oxide
NABI	North American Biotechnology Initiative
NAPPO	North American Plant Protection Organization
NEPA	National Environmental Policy Act
NHPA	National Historic Preservation Act
NMFS	National Marine Fisheries Service
NOP	National Organic Program
NRCS	National Resources Conservation Service

ACRONYMS, CONTINUED

OECD	Organization for Economic Cooperation and Development
OSTP	Office of Science and Technology Policy
PAT	phosphinothricin-acetyl-transferase
PBO	Plant Biosafety Office
PGF	pollen-mediated gene flow
PPA	Plant Protection Act
RR	Roundup Ready (registered trademark)
RSPM	Regional Standards for Phytosanitary Measures
TBW	tobacco budworm
TES	threatened and endangered species
TSCA	Toxic Substances Control Act
U.S.C.	United States Code
USDA	U.S. Department of Agriculture
USFWS	U.S. Fish and Wildlife Service
WPS	worker protection standard

1 PURPOSE AND NEED

1.1 REGULATORY AUTHORITY

“Protecting American agriculture” is the basic charge of the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS). APHIS provides leadership in ensuring the health and care of plants and animals. The agency improves agricultural productivity and competitiveness, and contributes to the national economy and the public health. USDA asserts that all methods of agricultural production (conventional, organic, or the use of genetically engineered varieties) can provide benefits to the environment, consumers, and farm income.

Since 1986, the United States government has regulated genetically engineered (GE) organisms pursuant to a regulatory framework known as the Coordinated Framework for the Regulation of Biotechnology (Coordinated Framework) (51 FR 23302, 57 FR 22984). The Coordinated Framework, published by the Office of Science and Technology Policy, describes the comprehensive federal regulatory policy for ensuring the safety of biotechnology research and products and explains how federal agencies will use existing Federal statutes in a manner to ensure public health and environmental safety while maintaining regulatory flexibility to avoid impeding the growth of the biotechnology industry. The Coordinated Framework is based on several important guiding principles: (1) agencies should define those transgenic organisms subject to review to the extent permitted by their respective statutory authorities; (2) agencies are required to focus on the characteristics and risks of the biotechnology product, not the process by which it is created; (3) agencies are mandated to exercise oversight of GE organisms only when there is evidence of “unreasonable” risk.

The Coordinated Framework explains the regulatory roles and authorities for the three major agencies involved in regulating GE organisms: USDA’s Animal and Plant Health Inspection Service (APHIS), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA).

APHIS is responsible for regulating GE organisms and plants under the plant pest authorities in the Plant Protection Act of 2000, as amended (7 USC § 7701 *et seq*.) to ensure that they do not pose a plant pest risk to the environment.

The FDA regulates GE organisms under the authority of the Federal Food, Drug, and Cosmetic Act (FFDCA). The FDA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those that are genetically engineered. To help developers of food and feed derived from GE crops comply with their obligations under Federal food safety laws, FDA encourages them to participate in a voluntary consultation process. All food and feed derived from GE crops currently on the market in the United States have successfully completed this consultation process. The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992 (57 FR 22984-23005). Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food.

The EPA regulates plant-incorporated protectants under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and certain biological control organisms under the Toxic Substances Control Act (TSCA). The EPA is responsible for regulating the sale, distribution and use of pesticides, including pesticides that are produced by an organism through techniques of modern biotechnology.

1.2 REGULATED ORGANISMS

The APHIS Biotechnology Regulatory Service's (BRS) mission is to protect America's agriculture and environment using a dynamic and science-based regulatory framework that allows for the safe development and use of GE organisms. APHIS regulations at 7 Code of Federal Regulations (CFR) Part 340 (hereafter Part 340), which were promulgated pursuant to authority granted by the PPA, regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the Plant Protection Act or to the regulatory requirements of Part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under Part 340 if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation (7 CFR 340.2) and is also considered a plant pest. A GE organism is also regulated under Part 340 when APHIS has reason to believe that the GE organism may be a plant pest or APHIS does not have information to determine if the GE organism is unlikely to pose a plant pest risk.

A person may petition the agency that a particular regulated article is unlikely to pose a plant pest risk, and therefore, is no longer regulated under the plant pest provisions of the Plant Protection Act or the regulations at 7 CFR 340. The petitioner is required to provide information under §340.6(c)(4) related to plant pest risk that the agency may use to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. A GE organism is no longer subject to the regulatory requirements of 7 CFR Part 340 or the plant pest provisions of the Plant Protection Act when APHIS determines that it is unlikely to pose a plant pest risk.

1.3 PETITION FOR DETERMINATION OF NONREGULATED STATUS: BAYER CROPSCIENCE LEPIDOPTERAN-RESISTANT AND GLUFOSINATE AMMONIUM-TOLERANT (TWINLINK™) COTTON, EVENTS GHB119 AND T304-40

Bayer Crop Science (BCS) has submitted a petition (APHIS Number 08-340-01p) seeking a determination that TwinLink™ Cotton Events GHB119 and T304-40 are unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under regulations at 7 CFR Part 340.

TwinLink™ Cotton is a combined-trait cotton developed using conventional breeding techniques to link two deoxyribonucleic acid (DNA) transformation events; each developed using DNA recombinant techniques. By crossing BCS' Cry1Ab Cotton (event T304-40) with BCS' Cry2Ae Cotton (event GHB119), BCS has developed a cotton resistant to lepidopteran pests. The

TwinLink™ Cotton also expresses a glufosinate ammonium herbicide tolerance trait based on LibertyLink® technology.

TwinLink™ Cotton is currently regulated under 7 CFR Part 340. Interstate movement, importation, and field testing of TwinLink™ Cotton have been conducted under notifications acknowledged by APHIS.

1.4 PURPOSE OF PRODUCT

BCS has developed the TwinLink™ Cotton as an alternative insect-resistant and herbicide-tolerant cotton product.

BCS has developed upland or Mexican cotton (*Gossypium hirsutum*) plants that express two insecticidal crystalline proteins, Cry1Ab and Cry2Ae, derived from the common soil bacterium, *Bacillus thuringiensis* (*Bt*). The Cry1Ab and Cry2Ae proteins in TwinLink™ Cotton are effective in controlling lepidopteran larvae such as bollworm (CBW, *Helicoverpa zea*), tobacco budworm (TBW, *Heliothis virenscens*), and fall armyworm (FAW, *Spodoptera frugiperda*) which are common pests of cotton. The rationale for using two *Bt* genes is that target insects are much less likely to develop resistance to both proteins simultaneously than to develop resistance to one toxic protein. In addition to the Cry1Ab and Cry2Ae proteins, the TwinLink™ Cotton contains the modifying phosphinothricin-acetyl-transferase (PAT) enzyme, encoded by the *bar* gene which confers tolerance to glufosinate ammonium-based herbicides. The *bar* gene, derived from *Streptomyces hygroscopicus*, encodes the PAT enzyme which acts to convert glufosinate ammonium into its inactive form, thus rendering the plant tolerant to the herbicide. This is the same enzyme that is expressed in BCS LibertyLink® Cotton (LLCotton25) that also confers tolerance to glufosinate ammonium herbicides.

Cotton producers in the U.S. are among the most technically advanced in the world, annually harvesting about 17 million bales or 7.2 billion pounds of cotton (US-EPA, 2009). In 2010, the USDA estimates that 18.9 million bales were harvested (USDA-NASS, 2010a) with approximately 10.9 million acres planted (USDA-NASS, 2010b). Cotton growers strive for high yields of high-value cotton fiber at the lowest production cost. To enhance successful cotton fiber production, the cotton industry relies heavily on pest control measures to control both weeds and lepidopteran insects. Infestation of cotton by lepidopteran larvae represents the largest insect threat to cotton production in the U.S. (Scott, 2008). To combat these threats and enhance production, cotton growers in the U.S. have adopted genetically improved cotton, to reduce pest management costs, obtain greater yields, and decrease pesticide costs; thereby producing greater grower return on investment (Scott, 2008). In 2010, 78% of all cotton grown in the U.S. contained herbicide-tolerant traits and 73% expressed insect-resistant traits (USDA-ERS, 2010a).

Cotton producers are currently limited to insect-resistant cotton varieties containing three *Bacillus thuringiensis* Cry1 endotoxin protein-based plant incorporated protectants. Each is deregulated by the USDA and registered by the EPA under FIFRA. The three proteins are Cry1Ac (Bollgard)—a combination of CrylAc and Cry2Ab2 (Bollgard II), Cry1F, and Cry1Ac (Widestrike) (USDA-APHIS, 2010a). These transgenic varieties offer almost complete protection against tobacco budworm, but may require additional applications of insecticide for control of cotton bollworm. Hence, despite the wide adoption of these Bt cotton varieties to control lepidopteran insects, these same species continue to be the most economically important pests of the crop. In 2009, it was estimated that the Heliothine complex of tobacco budworm and cotton bollworm infested 4.3 million acres of cotton and reduced yields across the Cotton Belt by 91,119 bales (Williams, 2010). Total 2009 cost and loss estimates for arthropod cotton pests were $502 million dollars (Williams, 2010).

TwinLink™ Cotton has been developed by BCS as an alternative insect-resistant and herbicide-tolerant cotton. The introduction of the Bt protein Cry2Ae provides an additional measure of insect protection not currently available in cotton. The tolerance to glufosinate ammonium, in addition to the insect resistance provided by the two Cry proteins, will provide cotton growers with a new approach for managing lepidopteran pests of cotton, as well as herbicide-resistant weeds.

Current insect resistance management strategies have delayed the development of resistance to Bt toxins. However, in a review of GE crops, Lemaux (2009) reports that some cotton lepidopteran-insect resistance to Bt toxins has occurred. The availability of additional lepidopteran insect-resistant traits in cotton, such as that conferred by incorporation of the Cry2Ae protein, further reduces the likelihood of pest resistance to existing pest control methods (Gould, 2003). A recent study evaluated several cultivation practices which might delay the development of insect resistance, including the planting of refuge areas with crops not engineered to contain Bt (Tabashnik et al., 2008).

Glufosinate ammonium tolerance was introduced by Bayer in its LibertyLink® Cotton to provide cotton growers with an alternative herbicide choice. The LibertyLink® Cotton product was deregulated by APHIS in 2003 (USDA-APHIS, 2003) and is widely available. EPA has published a voluntary pesticide resistant management labeling guideline providing guidance on reducing or managing the potential for herbicide-resistant weeds or volunteers (Pesticide Registration Notice 2001-5) (US-EPA, 2001a). Glufosinate ammonium tolerance provides growers with another herbicide option in cotton cultivation.

TwinLink™ Cotton, either alone or when combined by traditional breeding with other genetically-modified insect-resistant cotton varieties, by incorporating a new Cry protein, provides growers with an additional pest management option for lepidopteran-insect pest control and may contribute to a reduction in the likelihood of insect resistance to Bt insect-resistant cotton varieties. Specifically, BCS indicates that the use of TwinLink™ Cotton will provide:

1. Improved efficacy in reduction of major insect pests of cotton, independent of weather conditions.
2. Combined Bt trait product for insect resistance management.
3. Additional weed control options.
4. Excellent human, animal, non-target organism, and environmental safety profile.
5. Potentially reduced pesticide use.
6. Potentially enhanced yield.
7. Potentially decreased pest management costs.

1.5 APHIS RESPONSE TO PETITION FOR NONREGULATED STATUS

Under the authority of the plant pest provisions of the Plant Protection Act and 7 CFR Part 340, APHIS has issued regulations for the safe development and use of GE organisms. As required by 7 CFR 340.6, APHIS must respond to petitioners who request a determination of the regulated status of GE organisms, including GE plants such as BCS TwinLink™ Cotton. When a petition for nonregulated status is submitted, APHIS must make a determination if the GE organism is unlikely to pose a plant pest risk. If APHIS determines based on its Plant Pest Risk Assessment (PPRA) that the genetically engineered organism is unlikely to pose a plant pest risk, the genetically engineered organism is no longer subject the plant pest provisions of the Plant Protection Act and 7 CFR part 340.

TwinLink™ Cotton has been field tested in the U.S. since 2005, as authorized by APHIS. Data were provided in the petition for field trials completed prior to the petition submission. Field test reports can be found in the BCS TwinLink™ Cotton petition in Appendix 1, p.102 (Scott, 2008).

BCS has conducted 66 field trials under field release authorizations granted by USDA APHIS in diverse regions of the U.S., including Arizona, California, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Texas. Field tests conducted under APHIS oversight allow for evaluation in agricultural settings under confinement measures designed to minimize the likelihood of persistence in the environment after completion of the field trial. Under confined field trial conditions, data are gathered on multiple parameters and used by applicants to evaluate agronomic characteristics and product performance. These data are also valuable to APHIS for assessing the potential for a new variety to pose a plant pest risk. The data evaluated for TwinLink™ Cotton may be found in the document *Determination of Plant Pest Risk for Insect-Resistant and Glufosinate Ammonium-Tolerant (TwinLink™) Cotton, Gossypium hirsutum, Events T304-40 x GHB119* (USDA-APHIS, 2010b).

APHIS has prepared this environmental assessment to consider the potential environmental effects of an agency determination of nonregulated status consistent with Council of Environmental Quality's (CEQ) regulations implementing NEPA (40 CFR Parts 1500-1508), and the USDA and APHIS NEPA implementing regulations and procedures (7 CFR 1b, and 7 CFR Part 372) and the USDA and APHIS NEPA implementing regulations and procedures. This EA has been prepared in order to specifically evaluate the effects on the quality of the human environment[1] that may result from the deregulation of BCS TwinLink™ Cotton.

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[1] Under NEPA regulations, the "human environment" includes "the natural and physical environment and the relationship of people with that environment" (40 CFR §1508.14).

1.6 COORDINATED FRAMEWORK REVIEW

Food and Drug Administration

TwinLink™ Cotton (events T304-40 x GHB119) is within the scope of the 1992 FDA policy statement concerning regulation of products derived from new plant varieties, including those developed through biotechnology (US-FDA, 1992).

Bayer has provided the FDA with information on the identity, function, and characterization of the genes, for TwinLink™ Cotton, including expression of the gene products. The FDA is currently reviewing the information submitted by the applicant.

APHIS considers the FDA food and feed safety and nutritional assessment determination when assessing potential impacts that may result from the deregulation of a GE organism. In the absence of a completed FDA determination, APHIS takes into consideration prior FDA reviews of comparable products to make a preliminary assessment of the potential impacts.

Note that with regard to the expression of glufosinate ammonium tolerance, the gene construct in the Bayer TwinLink™ Cotton is the same as that approved by the FDA in June 2003 for the LibertyLink® Cotton product (US-FDA, 2003). In that approval, the FDA noted that the transformational event in LibertyLink® Cotton was not materially different in composition, safety, or any other relevant parameter in cotton grown, marketed, and consumed at that time (US-FDA, 2003). This previous FDA review will be used by APHIS to analyze the food and safety impacts associated with the incorporation and expression of glufosinate ammonium tolerance in TwinLink™ Cotton.

The FDA's oversight of the food and safety impacts associated with the incorporation and expression of pesticidal substances, in this case, the Cry proteins associated with Bt, are more limited. As discussed below, the EPA is the primary authority for the review of plant-incorporated protectants.

Environmental Protection Agency

The EPA has authority over the use of pesticidal substances and plant-incorporated protectants under the FIFRA as amended (7 USC §136, *et seq.*) and the FFDCA (21 USC §301, *et seq.).* EPA is currently reviewing information submitted by the applicant on the efficacy and potential environmental concerns associated with the use of this product. APHIS considers the EPA's regulatory assessment when assessing potential impacts that may result from the deregulation of a GE organism. In the absence of a completed EPA determination, APHIS takes into consideration prior EPA reviews of comparable products to make a preliminary assessment of the potential impacts.

Note that EPA has issued a tolerance exemption for Cry1Ab protein in all crops (40 CFR §174.511; US-EPA, 2010d), as well as for the PAT protein (40 CFR §174.522; US-EPA, 2010g). A temporary exemption from the requirement of a tolerance has been issued for Cry2Ae (40 CFR §174.530; US-EPA, 2010e). These previous EPA reviews will be used by APHIS to analyze the food and safety impacts associated with the incorporation and expression of the Cry proteins in TwinLink™ Cotton.

In addition to review of the crop with plant-incorporated protectants, EPA has authority under FIFRA to establish pesticide use restrictions; these use restrictions are presented on pesticide labels which are prepared during the pesticide registration process. The development of a transformed cotton crop providing tolerance to glufosinate ammonium may require a change in the EPA-approved label for this herbicide.

The current glufosinate ammonium label provides for its use on transformed crops expressing resistance to glufosinate ammonium, and specifically references products marketed under the trade name "LibertyLink" (BCS, 2010a). Glufosinate ammonium-tolerant cotton was first available in the U.S. with the introduction of LibertyLink® Cotton in 2004. Although the glufosinate ammonium tolerance trait expressed in TwinLink™ cotton is the same as that expressed in the LibertyLink® Cotton, the EPA is expected to publish a new label for glufosinate ammonium that also references the TwinLink™ product varieties. APHIS will use current glufosinate label as the basis for its evaluation of the potential impacts associated with the use of and exposure to glufosinate ammonium.

1.7 PUBLIC INVOLVEMENT

APHIS routinely seeks public comment on draft EAs prepared in response to petitions to deregulate GE organisms. APHIS does this through a notice published in the Federal Register. The issues discussed in this EA were developed by considering public concerns, as well as issues raised in public comments submitted for other environmental assessments of GE organisms, concerns raised in lawsuits, as well as those issues that have been raised by various stakeholders. These issues, including those regarding the agricultural production of cotton using various production methods, and the environmental and food/feed safety of GE plants, were addressed to analyze the potential environmental impacts of TwinLink™ Cotton.

This EA, the petition submitted by BCS, and APHIS's *Determination of Plant Pest Risk for Insect-Resistant and Glufosinate Ammonium-Tolerant (TwinLink™) Cotton, Gossypium hirsutum, Events T304-40 x GHB119* (USDA-APHIS, 2010b) will be available for public comment for a period of 60 days (7 CFR §340.6(d)(2)). Comments received by the end of the 60-day period will be reviewed and used to inform APHIS's determination decision of the regulated status of TwinLink™ Cotton and to assist APHIS in determining whether an Environmental Impact Statement is required prior to a determination of the regulated status of TwinLink™ Cotton.

1.8 ISSUES CONSIDERED

As stated above, the issues considered in this EA were developed based on APHIS's determination to deregulate certain GE organisms; and, for this particular EA, the specific deregulation of TwinLink™ Cotton. These issues include:

Cotton Production:

- Acreage and Areas of Cotton Production
- Cropping Practices
- Seed Production

- Organic Farming
- Specialty Cotton Production

Environmental Considerations:

- Water Resources
- Soil
- Air Quality
- Climate Change
- Animals
- Plants
- Biological Diversity
- Gene Movement

Public Health Considerations:

- Human Health
- Worker Safety

Animal Feed

Socioeconomic Issues:

- Domestic Economic Environment at Risk
- Trade Economic Environment at Risk
- Social Environment at Risk

Other Cumulative Effects

Threatened and Endangered Species

Other U.S. Regulatory Approvals and Compliance with Other Laws

2 AFFECTED ENVIRONMENT

The Affected Environment Section provides an overview of the use and biology of cotton, followed by a discussion of the current conditions of those aspects of the human environment potentially impacted by the deregulation of TwinLink™ Cotton. For the purposes of this draft EA, those aspects of the human environment are: cotton production practices, the physical environment, biological resources, public health, animal feed, and socioeconomic issues.

2.1 COTTON USE AND BIOLOGY

2.1.1 Cotton Taxonomy

Cotton is a perennial plant that is cultivated as an annual crop (USDA-ERS, 2009). The *Gossypium* genus is made up of approximately 50 species, but only five species are cultivated worldwide (OECD, 2008). The cultivated species upland cotton (*G. hirsutum*) represents 97% of cotton crop grown in the U.S. (USDA-ERS, 2009). The balance of U.S.-grown cotton is American Pima, the generic name for extra-long staple (ELS) cotton, *G. barbadense* (Meyer et al., 2007; USDA-ERS, 2009). ELS is cultivated primarily in irrigated regions of California, New Mexico, Arizona, and Texas (Meyer et al., 2007; USDA-ERS, 2009). The three other cultivated species are *G. arboretum, G. herbaceum,* and *G. lanceolatum* (OECD, 2008; Scott, 2008).

Seven genomes, designated A, B, C, D, E, F, and G, are found in the *Gossypium* genus (Endrizzi et al., 1984). Diploid (i.e., two sets of chromosomes) species (2n=26) are found on all continents, and a few are of some agricultural importance. The A genome is restricted in diploids to two species (*G. arboreum* and *G. herbaceum*) of the Old World. The D genome is restricted in diploids to some species of the New World, such as *G. thurberi*. The most important agricultural cottons, *G. hirsutum* and *G. barbadense,* are both allotetraploids (i.e., hybrids containing 4 complete haploid chromosome sets representing the complete chromosome sets from both parent organisms) (2n=4x=52). These cultivated varieties are of New World origin and are presumed to be the result of an ancient interspecific hybridization between Old World A genomes and New World D genomes (Scott, 2008). How and when the original crosses occurred has been subject to much speculation. Euploids (i.e., organisms containing equal numbers of the haploid chromosome sets) of these plants have 52 somatic chromosomes and are frequently designated as AADD (they behave as disomic polyploids, i.e., have two complete sets of chromosomes from both original ancestors). The New World allotetraploids are peculiar in the genus, because the species, at least in their wild forms, grow near the ocean as invaders in the constantly disturbed habitats of strand and associated environs. It is from these "weedy" or invader species that the cultivated cottons developed (Fryxell, 1984).

Due to the difference in ploidy level (i.e., number of sets of chromosomes), *G. hirsutum* cannot cross with wild diploid cottons. *G. hirsutum* is readily cross-compatible, i.e., can hybridize, only with other tetraploid members of the tribe *Gossypium*, which include *G. tomentosum* in Hawaii, *G. darwinii* in the Galapagos Islands, *G. mustelinum* in northeastern Brazil, *G. hirsutum* and *G. lanceolatum* in tropical/subtropical America, and *G. barbadense* in South America, as well as cultivated forms of *G. hirsutum* and *G. barbadense* (Fryxell, 1984).

These distinctions in genetic construct are important to recognize, as the genetic differences directly affect the ability of cultivated cotton to interbreed with wild cotton species as well as wild or feral cotton. There are only two wild cotton species found in the U.S.: *G. thurberi* (desert cotton), found in Arizona; and *G. tomentosum* (Hawaiian cotton), found in Hawaii (ITIS, 2010; USBG, 2010; USDA-ARS, 2010a). *G. tomentosum* has been crossed with *G. hirsutum* in breeding programs; however, no commercial cotton is produced in Hawaii (Scott, 2008; USDA-NASS, 2010c). Feral populations of upland cotton (*G. hirsutum*) can be found in southern Florida (USDA-FS, 2010). These populations are apparently self-sustaining because there is no commercial production of cotton in this region (Scott, 2008; USDA-NASS, 2010c).

2.1.2 Cotton Use

Cotton (*Gossypium* spp.) is a member of the Malvaceae family and is the world's most widely grown fiber crop, accounting for over 40% of the total world fiber use (Meyer et al., 2007). Cotton has been cultivated for its fiber for several thousand years (Scott, 2008). Other cotton products, such as cottonseed oil, cake, and cotton linters, are by-products of cotton fiber production (Scott, 2008).

2.2 AGRICULTURAL PRODUCTION OF COTTON

2.2.1 Acreage and Areas of Cotton Production

Cotton is cultivated mostly in subtropical and warm temperate zones (OECD, 2008). Temperature is the main climatic factor determining the geographic range in which cotton can be grown, but cultivation is also dependent upon high light intensity, good soil moisture, and soil fertility (Meyer et al., 2007; OECD, 2008).

Cotton is mainly produced in China, India, U.S., Pakistan, Brazil, and Uzbekistan, with these five countries contributing nearly 83% of world production (Table 2-1) (USDA-FAS, 2010a). The total planted area throughout the world in 2007-2008 was 33.2 million hectares, for a production of 116 million bales (approximately 26 million metric tons) (USDA-FAS, 2010a). The U.S. is the third largest producer of cotton fiber.

Table 2-1: Cotton Fiber: World Production in Select Countries, 2006 to 2010/11 (estimated) (1,000 metric tons)

	Year				
Country	**2006/2007**	**2007/2008**	**2008/2009**	**2009/2010**	**2010/2011 (est.)**
China	7,729	8,056	7,991	6,967	6,858
India	4,746	5,225	4,921	5,051	5,661
United States	4,700	4,182	2,790	2,654	4,109
Pakistan	2,090	1,872	1,894	2,090	2,025
Brazil	1,524	1,602	1,193	1,165	1,524
Uzbekistan	1,165	1,165	1,002	849	1,045
Australia	294	139	327	386	718
Other	4,258	3,814	3,210	2,907	3,463
Total	26,507	26,056	23,326	22,070	25,404

Source: (USDA-FAS, 2010a)

In the U.S. for the 2010 production year, cotton was grown on 4.4 million hectares (approximately 10.8 to 10.9 million acres) (USDA-NASS, 2010b). In the U.S., cotton is produced in 17 southern states identified as the Cotton Belt: Alabama, Arizona, Arkansas, California, Florida, Georgia, Kansas, Louisiana, Mississippi, Missouri, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia (Meyer et al., 2007; USDA-NASS, 2010c). The major cotton producing states are Texas (5.4 million acres), Georgia (1.3 million acres), Arkansas (0.540 million acres), Mississippi (0.420 million acres), North Carolina (0.545 million acres), and Tennessee (0.387 million acres) (USDA-NASS, 2010c).

Cotton acreage in the U.S. rose slightly during the first half of the 2000s, continuing a multi-decade trend. In the 1970s and 1980s, the area planted for cotton averaged about 12 million acres (USDA-ERS, 2009). The area planted rose to about 14 million acres in the 1990s and averaged over 14.5 million acres during the first half of the 2000s, representing an increase of 20% over the 1970s and 1980s acreage (USDA-ERS, 2009). Since 2006, however, U.S. cotton planted acreage has been considerably lower, as prices have favored the planting of alternative crops such as corn and soybeans (USDA-ERS, 2009). All cotton growing regions have experienced significant declines compared with the first half of the 2000s (USDA-ERS, 2009).

GE varieties of cotton have been very rapidly adopted. In 1996, when Bt cotton was first commercially introduced, initial plantings were estimated at 1.7 million acres (Benbrook, 2009). Of the approximate 11 million acres planted in cotton in 2010, about 93% (10.2 million acres) were GE cotton. Of this, 73% of the GE cotton acreage was GE insect-resistant (Bt) cotton, and 78% was herbicide-tolerant (USDA-ERS, 2010a).

2.2.2 Cropping Practices: Crop Rotation and Pesticide Use

Growers can choose from many cultivars of cotton marketed by companies that produce seed, including GE varieties (USDA-AMS, 2010a). Planting of cotton occurs after any danger of frost has passed and soil temperatures are at least 14^{o}C (57^{o}F) (OECD, 2008). Planting dates vary depending on the region, but generally range from early March in southern Texas to early May or June in the northern areas of the Cotton Belt. Cotton requires 180 to 200 days from planting to maturity (OECD, 2008). Therefore, cotton planted in March or April is ready for harvest in September.

Crop rotations (successive planting of different crops on the same land) are used to optimize soil nutrition and fertility and reduce pathogen loads (Gazaway et al., 2007; University of California IPM Online, 2008a). Cotton is often rotated with other crops in order to control various cotton pests including nematodes, verticillium wilt, seedling diseases, and pink bollworm (University of California IPM Online, 2008a). Rotation crops may include small grains, cowpea, corn, sorghum, alfalfa, onions or garlic, and nematode-resistant tomatoes. Rotations may last for two or three years. Winter cover crops are also utilized in cotton. These cover crops are used to provide winter soil cover and protection, build soil nitrogen and organic matter, reduce nitrogen leaching, suppress weeds, and provide a habitat for beneficial predatory and parasitic insects and spiders (Guerena and Sullivan, 2003).

Cotton production in the U.S. is highly mechanized and involves the extensive use of agronomic inputs and technology (USDA-APHIS, 2010a). The cost of production includes annual direct costs (seed, fertilizer, chemicals, irrigation, etc.), annual fixed costs (depreciation on equipment), and annual land rent (Hogan et al., 2005). These costs will vary depending on region and practices used. In 2009, average per acre costs for U.S. cotton growers for cotton seed, fertilizer (commercial fertilizers, soil conditioners, and manure), and chemicals (including pesticides and herbicides) accounted for 54% of the total dollars spent per planted acre or $232 per acre (USDA-ERS, 2010b).

Worldwide, cotton has proven vulnerable to the attack of many insect species. In the U.S., the cotton industry has consistently relied heavily on insecticide use strategies to manage arthropod pests (Gianessi and Carpenter, 1999). Resistance to commonly used insecticides (pyrethroids, organophosphates, and carbamates) has led to a need for new pesticide chemistries or other novel approaches, such as the use of sterile insects, pathogens, and transgenic cotton (Gianessi and Carpenter, 1999). Pest management in agricultural crop production is achieved through various management methods, including chemical (pesticides), cultural (mechanical cultivation, planting/harvesting dates, crop rotation, etc.), biological (antagonistic organisms), and bioengineering (primarily herbicide-tolerant and insect-resistant crops) (USDA-ERS, 2005a). GE insect-resistant cotton has been focused on incorporating *Bacillus thuringiensis* (*Bt*) Cry endotoxin protein based plant incorporated protectants (Stewart, 2007).

The most damaging insect pests of cotton attack the cotton square (the flower bud) or the cotton boll (the ovary containing developing seeds and fibers) (Gianessi and Carpenter, 1999). There are over 20 economically important pests of cotton. The major insect pests of U.S. cotton in 2009 are listed in Table 2-2 (Williams, 2010). Thrips (*Frankliniella occidentalis*), lygus (*Lygus hesperus*), and the budworm/bollworm complex each infested more than half of the U.S. cotton crop. The budworm/bollworm complex, which infested 56% of the acreage, was the third leading cause of yield loss due to insects. It is referred to as a complex because the larvae of these moth insects are identical when observed in the field (Gianessi and Carpenter, 1999). The complex consists of the cotton bollworm (*Helicoverpa zea*) and tobacco budworm (*Helicoverpa virescens*) whose small larvae feed on smaller squares and terminal buds, but whose older, larger larvae devour buds, flowers, and bolls, consuming both lint and seed (Gianessi and Carpenter, 1999). In 2009, the complex reduced yields by 0.486% or 91,119 bales of cotton (Williams, 2010). Bt cotton provides good control over bollworm and tobacco budworm, as well as loopers, fall armyworm, and beet armyworm (Stewart, 2007).

Table 2-2: Cotton Pests Ranked in Order of Bales Lost, Presenting Percent U.S. Cotton Yield Reduction, Number of Cotton Acres Infested, Percent of U.S. Cotton Infested, Bales Lost, in 2009

Pest	Percent U.S. Cotton Yield Reduction Attributed to Pest	Acres Infested with Pest	Percentage of Cotton Acreage Infested	Bales Lost
Thrips	0.713%	7,437,609	94.49%	138,207
Lygus	0.614%	4,209,086	53.47%	126,871
Bollworm/Budworm	0.486%	4,376,640	55.60%	91,119
Stink Bugs	0.371%	3,265,604	41.49%	79,327
Fall Armyworm	0.113%	1,835,299	23.32%	20,238

Pest	Percent U.S. Cotton Yield Reduction Attributed to Pest	Acres Infested with Pest	Percentage of Cotton Acreage Infested	Bales Lost
Spider Mites	0.099%	2,022,705	25.70%	21,673
Cotton Fleahoppers	0.093%	1,490,760	18.94%	15,598
Clouded Plant Bugs	0.038%	452,752	5.75%	8,300
Aphids	0.030%	3,851,114	48.93%	6,456
Silverleaf Whitefly (*Bemisia*)	0.008%	289,731	3.68%	2,245
Beet Armyworm	0.004%	703,067	8.93%	643
Other Insects	0.003%	62,193	0.79%	668
Boll Weevil	0.002%	116,247	1.48%	400
Grasshoppers	0.001%	1,128,763	14.34%	266
Banded Wing Whitefly	0.001%	524,519	6.66%	249
Cutworms	0.001%	386,007	4.90%	200
Loopers	0.000%	808,131	10.27%	12
Saltmarsh Caterpillars	0.000%	487,934	6.20%	0
Cotton Leaf Perforator	0.000%	2,585	0.03%	1
Pink Bollworm	0.000%	81,211	1.03%	0
European Cornborer	0.000%	655	0.01%	0
Southern Armyworms	0.000%	68,798	0.87%	0

Source: (Williams, 2010)

2.2.3 Seed Production

On an annual basis, certified seed of all varieties of cotton combined must be able to plant over 10 million acres in the U.S. alone (USDA-NASS, 2010b) This requires between 60,000 and 70,000 short tons of planting seed (National Cottonseed Products Association, 2011).

Public cotton breeding programs began in California in 1898 and private programs began in the early 1920s (Marra and Martin, 2007). Cotton was one of the earliest crops for which the Association of Official Seed Certifying Agencies (AOSCA) developed seed certification standards to maintain genetic purity of cotton varietal seed and specialty cotton crops by precluding gene flow and preventing cross pollination between species and varieties (AOSCA, 2010b; Wozniak, 2002).

In a seed certification program, classes of seed are identified to designate the seed generation from the original breeder source (Hartman and Kester, 1975). Foundation seed, Registered seed, and Certified seed production is controlled by public or private seed certification programs (see, e.g., AOSCA, 2010b). The original seed breeder seed stock is controlled by the developer of the variety (Adam, 2005; Hartman and Kester, 1975). The breeder stock is used to produce Foundation seed stock (Adam, 2005). The institution associated with the breeder controls the production of Foundation seed stock. Foundation seed stock, in turn, is used to produce Registered seed for distribution to licensees, such as seed companies (Adam, 2005). Registered seed is used by seed companies to produce large quantities of Certified seed (Adam, 2005;

Hartman and Kester, 1975). The Certified (or Select) seed is then sold to growers through commercial channels (Adam, 2005; Hartman and Kester, 1975).

Seed certification cultivation practices commonly include recommendations for minimum isolation distances between various seed lines and planting border or barrier rows to prevent pollen movement (Hartman and Kester, 1975; Wozniak, 2002). The isolation distance for Foundation, Registered, and Certified seeds, as dictated by the USDA Agricultural Marketing Service's (AMS) Federal Seed Act in 7 CFR Part 201.76, is 1,320, 1,320, and 660 feet, respectively (USDA-AMS, 2010c). During the growing season, seed certification agencies will monitor the fields for off-types, other crops, weeds, and disease (Wozniak, 2002). These certifying agencies also establish seed handling standards to reduce the likelihood of seed source mixing during production stages, including planting, harvesting, transporting, storage, cleaning, and ginning (Wozniak, 2002). Further discussion of cross-pollination, gene transfer, and weediness is presented in Subsection 2.4.4, Gene Movement.

2.2.4 Organic Farming

In the U.S., only products produced using specific methods and certified under the USDA National Organic Program (NOP) definition of organic farming can be marketed and labeled as “organic” (Ronald and Fouche, 2006; USDA-AMS, 2010b). The NOP is administered by USDA’s AMS.

Organic certification is a process-based certification, not a certification of the end product. The certification process specifies and audits the methods and procedures by which the product is produced (Ronald and Fouche, 2006). In accordance with NOP, an accredited organic certifying agent conducts an annual review of the certified operation’s organic system plan and makes on-site inspections of the certified operation and its records. Organic growers must maintain records to show that production and handling procedures comply with USDA organic standards.

The NOP regulations preclude the use of excluded methods. The NOP provides the following guidance under 7 CFR §205.105—

> To be sold or labeled as “100 percent organic,” “organic,” or “made with organic (specified ingredients or group(s)),” the product must be produced and handled without the use of:
>
> (a) Synthetic substances and ingredients,…
> (e) Excluded methods,…

Excluded methods are then defined at 7 CFR §205.2 as—

> A variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes and are not considered compatible with organic production. Such methods include cell fusion, microencapsulation and macroencapsulation, and recombinant DNA technology (including gene deletion, gene doubling, introducing a foreign gene, and changing the positions of genes when achieved by

> recombinant DNA technology). Such methods do not include the use of traditional breeding, conjugation, fermentation, hybridization, in vitro fertilization, or tissue culture.

The NOP has recognized the feasibility of protecting organically-produced crops from accidental contamination by GE crops by requiring that organic production plans include practical methods to protect organically-produced crops—

> Organic crops must be protected from contamination by prohibited substances used on adjoining lands (for example, drifting pesticides, fertilizer-laden runoff water, and pollen drift from genetically engineered…) (NCAT, 2003).

Organic farming operations, as described by the NOP, require organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with excluded methods from adjoining land that is not under organic management. Organic production operations must also develop and maintain an organic production system plan approved by their accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. In NOP organic systems, the use of GE crops, such as TwinLink™ Cotton, is excluded (USDA-AMS, 2010b).

The organic plan used as the basis for organic certification should include a description of practices used to prevent or reduce the likelihood of unwanted substances, like GE pollen or seed, at each step in the farming operation, such as planting, harvesting, storing, and transporting the crop (Krueger, 2007; Riddle, 2004). Organic plans should also include how the risk of GE pollen or co-mingling of seed will be monitored (Krueger, 2007; Riddle, 2004). Farmers using organic methods are requested to let neighboring farmers know that they are using organic production practices and request that the neighbors also help the organic farmer reduce contamination events (Krueger, 2007; NCAT, 2003).

Organic cotton provides price premiums for the grower (Guerena and Sullivan, 2003). Organic production begins with certified organically grown seed (Guerena and Sullivan, 2003). Certified organic cotton acreage is a relatively small percentage of overall cotton production in the U.S. The most recently available data show 15,377 acres of certified organic cotton production in 2008 (USDA-ERS, 2010c). This is 0.16% of the total 9.41 million acres of cotton planted in 2008 (USDA-ERS, 2009). Bayer CropScience FM 958, FM989, and ADF 2485 were the predominate varieties planted in 2010 by organic cotton producers (USDA-AMS, 2010b).

2.2.5 Specialty Cotton Production

Several specialty cottons are cultivated in the U.S., including ELS (*G. barbadense*) Pima varieties, specialty long-staple upland cotton (*G. hirustum*) Acala varieties, organic cotton, and naturally colored cotton (Lee, 1996; USDA-ERS, 2009). Historically, ELS cotton acreage has ranged from a low of 63,000 acres in 1983 to a high of 377,000 acres in 1989 (National Cotton Council of America, 2010). This represented 0.83% and 3.5% of the total cotton acreage planted for those years, respectively (National Cotton Council of America, 2010). Naturally colored cotton acreage was reported to be 4,000 acres in 1992 (Dickerson et al., 1999) and between 5,000 and 7,000 acres in 1995 (Lee, 1996).

Similar to the production of conventional seed, industry quality standards for specialty crop products have led these seed producers and growers to employ a variety of techniques to ensure that their products are not pollinated by or commingled with conventional or genetically engineered crops (Bradford, 2006). Common practices include maintaining isolation distances to prevent pollen movement from other cotton sources, planting border or barrier rows to intercept pollen, and employing natural barriers to pollen (NCAT, 2003; Wozniak, 2002). The Federal Seed Act Regulations provide additional details on Certified seed production (see 7 CFR 201, *et seq*). Field monitoring for off-types, other crops, weeds, disease etc. is also carried out by company staff and state crop improvement associations (Bradford, 2006). Seed handling standards are established by AOSCA to reduce the likelihood of seed source mixing during planting, harvesting, transporting, storage, cleaning, and ginning (AOSCA, 2004). In general, the conventional management practices used for conventional seed production are sufficient to meet standards for the production of specialty crop seed (Bradford, 2006).

2.3 PHYSICAL ENVIRONMENT

The use of fertilizers, pesticides, and water may affect segments of the environment including, but not limited to: waterways by increases in nutrient pollution; biodiversity because pesticide inputs cause species changes; the water table because of excessive irrigation practices; and productivity of cropland because irrigation increases salinity (Hoeft et al., 2000). Sediment, siltation, nutrients, pesticides, salinity, and pathogens are primary agricultural pollutants (USDA-ERS, 2005b).

2.3.1 Water Resources

Cotton has been developed as a drought-tolerant crop; cotton production water use varies according to the growing environment (Cotton Incorporated, 2010b). Successful cultivation of dryland (non-irrigated) cotton requires at least 500 millimeters (mm) (40 inches) of rainfall during the growing season (OECD, 2008). Cotton cultivated in the southwestern region of the U.S., including west Texas, southern New Mexico, southern Arizona, and southern California, requires irrigation (USDA-APHIS, 2010a). Where irrigation water is needed (approximately 35% of the U.S. cotton grown), cotton yields are also much higher (Cotton Incorporated, 2010a). The desert southwest requires a maximum of 40 inches of irrigation per year, although the humid southeast may only require about 18 inches of irrigation per year (Cotton Incorporated, 2010a). Carefully timing the application of irrigation water optimizes the plant’s vegetative growth, flowering, and boll production (OECD, 2008). The lack of affordable water has been noted as one factor in the reduction of acres of cotton grown in California, Arizona, and New Mexico in the past decade (Cotton Incorporated, 2010a). In these areas, cotton has been displaced by higher value crops and land uses (Cotton Incorporated, 2010a).

Conservation tillage and no-till practices have been shown to minimize surface water runoff and soil erosion and to improve soil quality by increasing the soil organic matter that helps bind soil nutrients and prevent their loss to runoff, erosion, and leaching (Leep et al., 2003). This decrease in soil erosion carries a corresponding decrease in non-point source surface water pollution of fertilizer and pesticides (Cotton Incorporated, 2010a, 2010c). Precision agriculture, a practice carefully managing nutrients and chemical applications, also ensures that inputs are used by the crop and are not entering ground or surface waters (Cotton Incorporated, 2010a). Precision agriculture practices may include computer models to predict water use based on plant growth stage, weather, soil moisture probe data collection, and thermal infrared imagery of plant leaf temperature (Cotton Incorporated, 2010a). Each of these practices has the potential to reduce soil-laden water runoff (USDA-NRCS, 2006b).

2.3.2 Soil

Cotton is cultivated in a wide variety of soils, but develops best in deep, arable soils with good drainage, high organic content, and a high moisture-retention capacity (OECD, 2008). Irrigation allows cultivation in poor-quality soils with necessary nutrients provided in the irrigation water (OECD, 2008).

Conventional tillage traditionally requires that the producer remove all plant residues and weeds from the soil surface prior to planting, and then continue to cultivate the soil while the crop is growing to control late emerging weeds (Cotton Incorporated, 2010c). This practice results in soil loss to wind and water erosion.

Conservation practices have been developed to reduce field tillage and thus reduce the corresponding soil loss (USDA-NRCS, 2006a). As conservation practices are adopted, there are increases in soil organic matter that helps bind soil nutrients and corresponding significant reductions over time in the loss of cropland soil from runoff, erosion, and leaching (Leep et al., 2003; USDA-NRCS, 2006a, 2006b). Total soil loss on highly erodible croplands and non-highly erodible croplands decreased from 462 million tons per year to 281 million tons per year or by 39.2% from 1982 to 2003 (USDA-NRCS, 2006b). This decrease in soil erosion carries a corresponding decrease in non-point source surface water pollution of fertilizer and pesticides (Cotton Incorporated, 2010a, 2010c). The reduction in soil erosion is also attributed to a decrease in the number of acres of highly erodible cropland being cultivated (USDA-NRCS, 2006b).

Over the last 10 years, cotton cultivation has contributed to this reduction in soil loss through a shift towards soil conservation practices (Cotton Incorporated, 2010c). Conservation practices adopted in cotton cultivation include use of seed treatment fungicides, herbicides, and herbicide-tolerant cotton varieties, each of which provide for the control of cotton disease and weeds with minimal tillage (Cotton Incorporated, 2010c; USDA-NRCS, 2006b). In the U.S., conservation tillage in cotton fields has increased from 0.5 million acres in 1990 to about 2.75 million acres in 2004; in 2008, approximately 67% of cotton growers reported adopting some form of conservation tillage (Cotton Incorporated, 2010c). Increases in total acres dedicated to conservation tillage have been attributed to an increased use of GE seed which eliminates the need for mechanical weed control (Alabama Cooperative Extension System, 1996; McClelland et al., 2000; Towery and Werblow, 2010; USDA-NRCS, 2006b).

By definition, conservation tillage leaves at least 30% of the soil covered by crop residue (Peet, 2001). The new crop is planted into the plant residue or in narrow strips of tilled soil.

This is in comparison to conventional tillage where the seedbed is prepared through plowing (to turn the soil surface over), disking (to reduce the size of soil clods created by plowing), and harrowing (to reduce the size of clods left by disking) (Peet, 2001). Benefits of reduced tillage practices include maintenance of soil organic matter and beneficial insects, increased soil water-holding capacity, less soil and nutrient loss from the field, reduced soil compaction, and less time and labor required to prepare the field for planting (Peet, 2001).

Weed control in conservation tillage cotton is primarily through the use of herbicides (Alabama Cooperative Extension System, 1996). Preplant "burndown" herbicides such as paraquat and glyphosate and some pre-emergent herbicides make up the primary weed control methods. Winter and cover crops are also utilized in conservation tillage for the purpose of suppressing weeds (Alabama Cooperative Extension System, 1996). Wheat and rye are commonly employed because of their ease in killing prior to cotton planting. The use of herbicide-resistant cotton has allowed cotton growers to more readily adopt soil conservation practices because it provides an economical, effective means of controlling weeds in post-plant cotton (Alabama Cooperative Extension System, 1996; McClelland et al., 2000).

In some regions, conservation tillage is required. Farmers, including cotton growers, producing crops on highly erodible land are required by law to maintain a soil conservation plan approved by the USDA National Resources Conservation Service (NRCS) (USDA-ERS, 2010d). These soil conservation plans are prepared by the grower pursuant to the 1985 Food Security Act Conservation Compliance and Sodbuster programs to minimize soil erosion (USDA-ERS, 2010d).

The adoption of Bt cotton, which may require reduced quantities of pesticides, may benefit soil quality by reducing the risks associated with environmental spills or misapplications of chemical insecticides to the soil, and reducing the frequency of application. Reduced insecticide applications also mean a reduction in the number of trips across the field with heavy farm equipment which contributes to soil compaction, especially when the soil is wet. The number of acre-treatments per year of budworm/bollworm insecticides declined from over 10 million to less than 3 million between the years 1996 and 2007 (USDA-NASS, 2008). This is attributed to the widespread adoption of Bt cotton, introduced commercially in 1996, which increased from 1.73 million acres of cotton planted to 7.6 million acres in the U.S. during the same time period (Benbrook, 2009).

2.3.3 Air Quality

Agricultural air emission sources include smoke from agricultural burning, vehicle exhaust associated with equipment used in tillage and harvest, suspended soil particulates associated with tillage, pesticide drift from spraying, and nitrous oxide emissions from the use of nitrogen fertilizer (Aneja et al., 2009; Hoeft et al., 2000; US-EPA, 2010b; USDA-NRCS, 2006c). These agricultural activities individually have the potential to cause negative impacts to air quality.

Aerial application of pesticides may cause air quality impacts from drift and diffusion. Pesticides may volatilize after application to soil or plant surfaces and may also move as constituents of entrained materials in wind eroded soils (Vogel et al., 2008).

Many of the conservation plans and practices being developed by cotton growers to comply with the Conservation Compliance and Sodbuster programs have an air quality focus which target reductions in air emissions from agricultural operations (Cotton Incorporated, 2010c; USDA-NRCS, 2006a). Practices to improve air quality include conservation tillage, residue management, wind breaks, road treatments, burn management, prunings shredding, feed management, manure management, integrated pest management, chemical storage, nutrient management, fertilizer injection, chemigation and fertigation (inclusion in irrigation systems), conservation irrigation, scrubbers, and equipment calibration (USDA-NRCS, 2006a). Conservation tillage practices resulting in improved air quality include: fewer tractor passes across a field, thus decreasing dust generation and tractor emissions; and an increase in surface plant residues and untilled organic matter which physically hold the soil in place and reduce wind erosion (Baker et al., 2005; Cotton Incorporated, 2010d; USDA-NRCS, 2006a). The USDA has estimated that by 2006, the adoption of conservation management plans in the San Joaquin Valley of California had reduced air emissions by 34 tons daily, or more than 20% of the total emissions attributed to agricultural practices (Baker et al., 2005; USDA-NRCS, 2006a).

2.3.4 Climate Change

Agriculture, including land-use changes for farming, is responsible for an estimated 6% of all human-induced greenhouse gases (GHG) in the U.S. (US-EPA, 2010b). Emissions of GHG released from agricultural equipment (e.g., irrigation pumps and tractors) include carbon monoxide, nitrogen oxides, reactive organic gases, particulate matter, and sulfur oxides (US-EPA, 2010b). Agricultural soil management practices, including nitrogen-based fertilizer application and cropping practices, are the largest source of U.S. nitrous oxide (N_2O) emissions; croplands account for 69% of the total N_2O emissions attributable to agricultural land uses (US-EPA, 2010b). Agriculture sources of methane emissions are primarily associated with enteric emissions of gas from cattle and manure management. Carbon dioxide is also a significant GHG gas associated with several agricultural practices, including land uses and energy consumption (US-EPA, 2010b).

Tillage contributes to the release of GHG because of the loss of carbon dioxide (CO_2) to the atmosphere, and the exposure and oxidation of soil organic matter (Baker et al., 2005). The carbon footprint for cotton production has been estimated to be approximately 300 pounds of carbon equivalent emission per acre (Nelson et al., 2009). The carbon footprint of cotton is directly affected by the associated cultivation practices. In a no-tillage production environment, if credit is taken for the sequestration of carbon in soil, cotton actually stores more carbon than is emitted from the production (Cotton Incorporated, 2010d). Reduced tillage increases sequestration rates of carbon emissions in soils, with the estimated increase to be as high as 400 pounds of carbon per acre per year (Causarano et al., 2006).

The contribution of agriculture to climate change is largely dependent on the production practices employed to grow various commodities, the region in which the commodities are grown, and the individual choices made by growers. For example, emissions of nitrous oxide, produced naturally in soils through microbial nitrification and denitrification, can be dramatically influenced by fertilization, introduction of grazing animals, cultivation of nitrogen-fixing crops and forage, retention of crop residues (i.e., no-till conservation), irrigation, and fallowing of land (US-EPA, 2010b). These same agricultural practices can influence the decomposition of carbon-containing organic matter sequestered in soil, resulting in conversion to carbon dioxide loss to the atmosphere (US-EPA, 2010b). Conversion of crop land to pasture results in an increase in carbon and nitrogen sequestration in soils (US-EPA, 2010b).

The EPA has identified regional differences in GHG emissions associated with agricultural practices on different soil types, noting that carbon emission rates differ between mineral soils and organic soils (US-EPA, 2010b). Mineral soils contain from 1 to 6% organic carbon by weight in their natural state, whereas organic soils may contain as much as 20% carbon by weight (US-EPA, 2010b). In mineral soils, up to 50% of the soil organic carbon can be released to the atmosphere on the initial conversion, however, over time, the soil establishes a new equilibrium that reflects a balance between carbon inputs from decaying plant matter and organic amendments and carbon losses from microbial decomposition (US-EPA, 2010b). Organic soils, with their depth and richness in carbon content, continue to release carbon to the atmosphere for a longer period of time (US-EPA, 2010b). The EPA has estimated that mineral soil-based cropland areas sequestered over 45.7 Tg CO_2 Eq[1] in 2008, as compared with carbon emissions from organic soil-based croplands of 27.7 Tg CO_2 Eq (US-EPA, 2010b). The adoption of conservation tillage, particularly in the Midwest regions with mineral soil shows the highest rates of carbon sequestration (US-EPA, 2010b).

Changes in agriculture-related GHG production will not be significant unless large amounts of crop plantings produce changes in measureable concentrations (USDA-APHIS, 2010a). For example, the EPA has identified a net reduction in the sequestration of carbon in soil over an 18-year time scale, which it attributes to the declining influence of the Conservation Reserve Program which had encouraged growers to take marginal lands out of production (US-EPA, 2010b). To a certain extent, the EPA also noted that adoption of conservation tillage resulted in increases in carbon sequestration on those croplands (US-EPA, 2010b). The highest rates of carbon sequestration in mineral soils occurred in the Midwest, which is the region with the largest area of cropland managed with conservation tillage (US-EPA, 2010b). This is in contrast to the highest emission rates from organic soils noted in the southeastern coastal region, the areas around the Great Lakes, and the central and northern agricultural areas along the West Coast (US-EPA, 2010b).

One outcome of the potential effects of agriculture on climate change is the potential effect of the climate change on agriculture. In response to climate change, the current range of weeds and pests of agriculture is expected to increase. Current agricultural practices will be required to change in response to these changes in the ranges of weeds and pests of agriculture (IPCC, 2007).

Climate change may potentially provide a positive impact to agriculture in general. The Intergovernmental Panel on Climate Change (IPCC) predicts that potential climate change in

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[1] The global warming potential of greenhouse gases are measured against the reference gas CO_2, and are reported as teragrams (or million metric tons) of CO_2 Equivalent, expressed as Tg CO_2 Eq.

North America may result in an increase in crop yield by 5-20% for this century (IPCC, 2007). However, this positive impact will not be observed across all growing regions. The IPCC report notes that certain regions of the U.S. will be negatively impacted because the available water resources may be substantially reduced. Note that the extent of climate change effects on agriculture is highly speculative. Nevertheless, North American production is expected to adapt to climate change impacts with improved cultivars and responsive farm management (IPCC, 2007).

2.4 ANIMAL AND PLANT COMMUNITIES

Modern conservation practices incorporated in cotton cultivation have brought a positive impact to animal and plant communities, through reduced tillage, more carefully controlled and targeted chemical placement (fertilizers and pesticides), and better control of irrigation systems. The GE crop systems provide opportunities to optimize the introduction and implementation of these practices, and have the potential to create more of these benefits. For example, herbicide tolerance allows cultivation with minimal tillage required to control volunteers and weeds (Towery and Werblow, 2010). This subsection provides an overview of the relationships of these practices to the biotic community.

2.4.1 Animals

Intensive agricultural lands, such as those used in crop production, usually have low levels of biodiversity compared with adjacent natural areas. Tillage, seed bed preparation, planting of a monoculture crop, pesticide use, fertilizer use, and harvest result in limited habitat and a correspondingly limited diversity of plants and animals (Lovett et al., 2003). However, the implementation of better cropland management strategies can increase the value of crop fields to wildlife (Sharpe, 2010). Some of these strategies are noted below.

- Conservation tillage and no-till practices have a positive impact on wildlife (Towery and Werblow, 2010). Benefits include improved water quality, retention of cover, availability of waste grain on the soil surface for feed, and increased populations of invertebrates as a food source for turkey, quail, and songbirds (Sharpe, 2010).

- Crop rotations reduce the likelihood of crop disease, insect pests, weed pests, and the need for pesticides (University of California IPM Online, 2008a). Reduced pesticide use has a direct positive effect on wildlife by reducing the direct exposure of birds, mammals, and fish to pesticides. Indirect benefits include less alteration of suitable wildlife habitat and an available food supply of insects for insectivores (Palmer and Bromley, 2010; Sharpe, 2010). Crop rotations with legumes and small grains have been shown to provide excellent wildlife nesting cover, food, and brood-rearing habitat for quail in North Carolina (Sharpe, 2010).

- Field edges can be managed to promote wildlife. These borders are often the least productive areas in a farm field and in some cases, the cost of producing crop areas along field edges exceeds the value of the crop produced (Sharpe, 2010). Allowing field edges to return to volunteer vegetation does not contribute to major pest problems in the crop field itself (Sharpe, 2010). Volunteer border vegetation, such as ragweed, goldenrod, asters, and forbs, quickly develops into nesting and brood habitat for quail and a multitude of songbirds (Sharpe, 2010). Research conducted at North Carolina State University and the North Carolina Wildlife Resources Commission found that quail populations doubled when field borders were used (Sharpe, 2010).

- Contour-strip cropping is another management practice that can be used to promote wildlife habitat. This practice alternates strips of row crops with strips of solid stand crops (i.e., grasses, legumes, or small grains) with the strips following the contour of the land (Sharpe, 2010). The primary purpose of contour-strip cropping is to reduce soil erosion and water runoff, but the solid stand crop also provides a nesting and roosting cover for wildlife (Sharpe, 2010).

- Drainage ditches, hedgerows, riparian areas, and adjacent woodlands to a cotton field also contribute to wildlife populations. Ditch banks, for example, function as narrow wetlands that provide nesting sites and cover, serve as wildlife corridors, and provide areas for the wildlife to occupy when crop fields lack cover (Sharpe, 2010). Ditches have been shown to support birds, rodents, reptiles, furbearers, amphibians, fish, and aquatic organisms (Sharpe, 2010). Minimizing pesticide exposure of ditches, aquatic habitats, border areas, strip-crop areas, and non-crop habitats may help protect fish and wildlife resources (Palmer and Bromley, 2010).

Although many of the invertebrate organisms found in cotton-producing areas are considered pests, such as the cotton bollworm and tobacco budworm, most invertebrates are considered beneficial (University of Arkansas Cooperative Extension Service, 2006). Beneficial insects include a wide variety of predators which catch and eat smaller insects and parasitic insects that live on or in the body of other insects during at least one stage of their life cycle. Other beneficial insects are the pollinators. Major pollinators of *G. hirsutum* are bumble bees (*Bombus spp*.), black bees (*Melissodes spp*.), and honey bees (*Apis mellifera*) (McGregor, 1976). Pollinators of *G. tomentosum* are principally moths (McGregor, 1976). Other beneficial organisms, including earthworms, termites, ants, beetles, millipedes, and others contribute to the decay of organic matter and the cycling of soil nutrients (Ruiz et al., 2008).

2.4.2 Plants

The landscape surrounding a cotton field varies depending on the region. In certain areas, cotton fields may be bordered by other cotton (or any other crop) fields or may also be surrounded by woodland, rangelands, and/or pasture/grassland areas. These plant communities may be natural or managed plant habitats for the control of soil and wind erosion and serve as wildlife habitats (USDA-APHIS, 2010a).

Weed control programs are important aspects of cotton cultivation. In this context, weeds are those plants which, when growing in the cotton field, compete with the cotton for space, water, nutrients, and sunlight, and may thus include native species (University of California IPM Online, 2009c). Weeds also interfere with harvesting equipment and can stain lint (USDA-APHIS, 2010a).

Cotton is susceptible to weeds because it is easily outgrown during its early season growth and total crop failure can occur if weeds are not properly controlled (Alabama Cooperative Extension System, 1996). Weed control typically involves an integrated approach that includes herbicides, crop rotation, weed surveillance, and weed monitoring (University of California IPM Online, 2009b). Crop rotation is an important component of a successful weed management program in cotton cultivation (University of California IPM Online, 2009b). If a crop is cultivated year after year in the same fields, with the same cultivation practices followed, there is a very good chance that one or more weed species will increase in these fields (University of California IPM Online, 2009b). It is important that cotton cultivation practices employ a variety of cultural practices, as well as chemical controls (University of California IPM Online, 2009b).

The types of weeds in and around a cotton field depend on the immediate area in which the cotton is planted. Those weed species also vary depending on the geographic region in which the cotton is planted (USDA-APHIS, 2010a). For example, California has over 50 common weeds found in cotton (University of California IPM Online, 2009b). Common weeds in cotton include annual and perennial grasses (monocots), broad-leaf weeds (dicots), and sedges (*Cyperus spp.*). To assist growers in managing weeds, individual states, typically through their state agricultural extension service, will list the prevalent weeds of a crop and what is the most effective means for their control. For example, common weeds in California cotton and recommended herbicidal control can be found at University of California IPM Online (2009b).

Herbicide-tolerant cotton provides several weed management advantages to the growers. Broad spectrum post-emergent herbicides, such as glufosinate ammonium, provide control of weeds early in the cultivation cycle, minimizing competition in the fields, and providing optimal conditions for cotton growth (University of California IPM Online, 2009b). Applications of over-the-top post-emergent broad spectrum herbicides to an herbicide-tolerant crop allow the grower to decrease the overall use of herbicides before cultivation, reduce use of soil-applied herbicides, and streamline field cultivation activities for weed control (Marra and Martin, 2007; O'Sullivan and Sikkema, 2004). Production costs decrease because growers apply fewer herbicides and field cultivation requirements are reduced (University of California IPM Online, 2009b).

Herbicide-tolerant cotton has the potential to impact the cultivation of other crops in the same fields in later years. Cotton is periodically seen as a volunteer in soybeans, either when cotton seeds have successfully overwintered or in fields where a failed cotton crop is replanted to soybeans (Stewart et al., 2003). Cotton volunteers are limited by the geography in which they are initially planted; cotton does not survive as a perennial where freezing temperatures are reached in the winter (Scott, 2008). These cotton volunteers typically do not reduce crop yield, but can act as reservoirs for insect pests of cotton (Stewart et al., 2003). Successful control of cotton volunteers, including herbicide-resistant varieties, is accomplished through using various combinations of herbicides (Miller et al., 2004; Stewart et al., 2003).

2.4.3 Biological Diversity

Biodiversity refers to all plants, animals, and microorganisms interacting in an ecosystem (Wilson, 1988). Among other benefits, biodiversity provides valuable genetic resources for crop improvement (Harlan, 1975) and also provides other functions beyond food, fiber, fuel, and income. These include pollination, genetic introgression, biological control, nutrient recycling, competition against natural enemies, soil structure, soil and water conservation, disease suppression, control of local microclimate, control of local hydrological processes, and detoxification of noxious chemicals (Altieri, 2000). The loss of biodiversity results in a need for costly external inputs in order to provide these functions to the crop (Altieri, 2000).

The degree of biodiversity in an agroecosystem depends on four primary characteristics: 1) diversity of vegetation within and around the agroecosystem; 2) permanence of various crops within the system; 3) intensity of management; and 4) extent of isolation of the agroecosystem from natural vegetation (Altieri, 1999). The reintroduction of woodlots, fencerows, hedgerows, wetlands, etc. is one way to reintroduce biodiversity into large scale monocultures. Some enhancement strategies include intercropping (the planting of two or more crops simultaneously to occupy the same field), agroforestry, crop rotations, cover crops, no-tillage, composting, green manuring (growing a crop specifically for the purpose of incorporating it into the soil in order to provide nutrients and organic matter), addition of organic matter (compost, green manure, animal manure, etc.), and hedgerows and windbreaks (Altieri, 1999). To some degree these practices are being utilized by cotton growers to increase biodiversity (Cotton Incorporated, 2010e).

The use of genetically modified cotton affects biological diversity by: 1) providing the opportunity to use ecological management practices that enhance habitat creation and 2) decreasing the use of pesticides which in turn, limits the impact on the floral and faunal communities that can potentially inhabit the fields and immediate surroundings. The use of no-till, cover crops, crop rotation, intercropping, and good ditch/border/hedgerow management contributes to biodiversity in and around cotton fields (Palmer and Bromley, 2010; Sharpe, 2010).

Habitat preservation and biodiversity, as well as cotton production, have benefited from modern cotton technology. It is now possible to grow 50% more cotton on the same land required 40 years ago (Cotton Incorporated, 2010e). Increased productivity on the same amount of land allows growers to preserve habitat while maintaining food and fiber security (Cotton Incorporated, 2010e). Various methods for promoting animal and plant biodiversity in and around cotton fields have already been discussed above. Habitat preservation and plant biodiversity have a large impact on insect populations. Research conducted by Altieri (1999) and Altieri and Letourneau (Altieri and Letourneau, 1982, 1984) indicates:

1) Maintaining some weeds harbors and supports beneficial arthropods that suppress herbivore insect pests;
2) Polycultures of plants support lower herbivore populations because they provide a more stable and continuous availability of food and habitat for beneficial insects; and
3) Adjacent wild vegetation provides alternate food and habitat for natural enemies to pest herbivores.

The use of broad-spectrum insecticides is one of the most severe constraints for biological diversity in crops (Cattaneo et al., 2006; Sisterson et al., 2007; USDA-APHIS, 2010a). One of the benefits of Bt cotton has been the reduction of broad-spectrum insecticide use during cotton production (Fernandez-Cornejo and Caswell, 2006).

However, there is concern over the non-target effects of transgenic cotton, especially Bt cotton, on beneficial insects and non-target pests. Research in Arizona has shown that Bt cotton has a minimum effect on both the number and function of foliar-dwelling arthropod natural enemies (Naranjo, 2005a, 2005b). In contrast, broad-spectrum insecticide use caused a 48% reduction in 13 of the 22 taxa that were studied (Naranjo, 2005a). A comparison of non-transgenic and transgenic cotton revealed no substantial impact on ant and beetle diversity in cotton fields, but broad-spectrum insecticide use considerably reduced this diversity (Cattaneo et al., 2006).

Soil microorganisms play a key role in soil structure formation, decomposition of organic matter, toxin removal, nutrient cycling, and most biochemical soil processes (Garbeva et al., 2004). They also suppress soil-borne plant diseases and promote plant growth (Doran et al., 1996). The main factors affecting microbial population size and diversity include plant type (providers of specific carbon and energy sources into the soil), soil type (texture, structure, organic matter, aggregate stability, pH, and nutrient content), and agricultural management practices (crop rotation, tillage, herbicide and fertilizer application, and irrigation) (Garbeva et al., 2004). Plant roots, including cotton, release a large variety of compounds into the soil creating a unique environment for microorganisms in the rhizosphere. Microbial diversity in the rhizosphere is extensive and differs from the microbial community in the bulk soil (Garbeva et al., 2004).

2.4.4 Gene Movement

The possibility of gene movement from the host plant into native or feral populations of *Gossypium* species or wild or weedy relatives of cotton has been raised as an issue of concern (US-EPA, 2010a). This subsection provides a basis for evaluating the weediness potential of cotton as well as the potential for outcrossing and gene escape in cotton. The potential for outcrossing or gene escape is defined as the ability of the gene to escape to wild cotton relatives.

G. hirsutum is a perennial plant that is cultivated as an annual in the U.S. (USDA-ERS, 2009). Cultivation as an annual means that the entire cotton plant is harvested at the end of each growing year, and new seed is planted in following years. Because of the structure of the cotton boll, cotton seed dispersal in the field is rarely successful (Scott, 2008). Pollen dispersal is the most likely potential source of gene movement (Scott, 2008).

The cotton flower is perfect, containing both male and female parts, and can self- or cross-pollinate (Meyer et al., 2007). However, the flowers of most cotton species, including *G. hirsutum*, are generally considered to be self-pollinating (Meyer et al., 2007; Scott, 2008).

Cotton pollen is heavy and somewhat sticky and does not lend itself to wind dispersal and subsequent pollination (Scott, 2008). Cotton can, however, be pollinated by insects. Bees — wild bees, honeybees (*Apis mellifera*), and bumblebees (*Bombus spp.*) — are the primary insect pollinators (Pleasants and Wendel, 2010). Bees collect mainly the nectar from the cotton plants, and rarely the pollen. In addition, physical isolation from plants attractive to the bees significantly reduces the potential for pollen movement, as cotton flowers have a nectar high in glucose and low in sucrose, which probably makes it less attractive to bees. McGregor (1976) traced the movement of pollen from a cotton field surrounded by a large number of honeybee colonies using fluorescent particles on source cotton plants. McGregor illustrated a rapid decline in the transference of cotton pollen over distance, and found that at 150 to 200 feet away from the source plant, only 1.6% of the bees showed the presence of the fluorescent particles. By comparison, the isolation distances for Foundation, Registered, and Certified seeds in 7 CFR Part 201 are 1320, 1320, and 660 feet, respectively (USDA-AMS, 2010c).

Despite these characteristics of cotton pollen which limit wind dispersal and bee pollination, it is common practice to maintain isolation distances to prevent pollen movement from other cotton sources and to plant border or barrier rows to intercept pollen to maintain and protect cotton seed characteristics for seed certification programs (Wozniak, 2002). Field monitoring for off-types, other crops, weeds, disease etc. is carried out by both growers and state crop improvement associations (AOSCA, 2010a). Seed handling standards are established by AOSCA to reduce the likelihood of seed source mixing during planting, harvesting, transporting, storage, cleaning, and ginning (Bradford, 2006).

A majority of field-based research with cotton shows an outcrossing rate of 10% or less within 1 meter of the pollen source (USDA-APHIS, 2010a). In upland cotton, outcrossing studies suggest that pollen carryover decreases very rapidly as the distance to the closest marker pollen row increases, and that very little pollen is transferred beyond 12 meters (McGregor, 1976; Scott, 2008; Vaissiere and Vinson, 1994).

Reports of outcrossing of a bromoxynil-tolerant cotton are described in the USDA petition for CalGene’s GlyTol cotton 06-332-01p and in a study of pollen mediated gene flow by Van Deynze et al. (2005). Seed samples were collected in the border rows of Calgene's winter nursery sites in Catamarca, Argentina, and Pongola, Republic of South Africa, as well as in Stoneville, MS, USA. Sampling distance was one to 20 meters away from the bromoxynil-tolerant cotton. The frequency of outcrossing was determined by the crop and the pollinator. It is interesting to note that although the rate is higher for Argentina and South Africa (most likely due to the behavioral differences between European and African honeybees), the pattern of decline with distance was the same. Van Deynze et al. (2005) measured pollen-mediated gene flow (PGF) in four directions over two years from commercial seed fields of bromoxynil-tolerant (BXN) and Roundup Ready® (RR) cotton in the California cotton growing region at various distances from non-transgenic cotton fields. Their results confirmed, as well as refined, those of Kareiva et al. (1994), as larger distances were studied. In spite of variations due to the respective cardinal positions of the fields, the same decline with distance was observed. Van Deynze et al. further reported that a 55-meter non-planted area was a sufficiently broad distance to maintain varietal purity (Van Deynze et al., 2005). This finding is consistent with the distance limitations identified by McGregor.

There are few wild relatives of cultivated cotton where cotton is cultivated. Two species of cotton are cultivated in the U.S.: upland cotton (*G. hirsutum*) and Pima cotton (*G. barbadense*) (USDA-ERS, 2009). There are two wild relatives of interest to U.S. growers: *G. thurberi* and *G. tomentosum,* found in the mountains of southern Arizona and in Hawaii, respectively. Outcrossing of genes from TwinLink™ Cotton to *G. thurberi* is unlikely to occur because *G.*

thurberi contains only the D genome whereas *G. hirsutum* contains both the A and D genome. The difference in chromosome numbers precludes sexual compatibility of *G. hirsutum* with *G. thurberi* (OECD, 2008).

G. tomentosum is native to the Hawaiian Islands, occurring primarily in arid, rock, or clay coastal plains (Pleasants and Wendel, 2010; USDA-APHIS, 2010a). *G. tomentosum* is not known to be weedy or to have invasive characteristics, and is considered a rare plant in Hawaii (Pleasants and Wendel, 2010; University of Hawaii at Manoa, 2001). In laboratory and greenhouse breeding programs with hand pollination, *G. tomentosum* and *G. hirsutum* are sexually compatible and form viable progeny (Pleasants and Wendel, 2010). However, DNA marker analyses have not found evidence of genes from *G hirsutum* occurring in native populations of *G. tomentosum* (DeJoode and Wendel, 1992). It is possible that the lack of evidence of movement of *G. hirsutum* genes into *G. tomentosum* is the result of lack of opportunity because cotton has not been grown commercially in Hawaii for at least the last 45 years (USDA-APHIS, 2010a).

Historic literature suggests that there are barriers to cross pollination between *G. tomentosum* and either *G. hirsutum* or *G. barbadense* based on different flowering patterns and pollinators (Pleasants and Wendel, 2010). Field and laboratory studies demonstrated that the historic literature is incorrect, and that these three species share common pollinators, and further that differences in flower structure and flowering habits do not serve as barriers to cross pollination (Pleasants and Wendel, 2010).

EPA recognizes a possibility of gene transfer from Bt cotton to feral cotton relatives in Hawaii, Florida, Puerto Rico, and the U.S. Virgin Islands (US-EPA, 2008a). Where feral populations of cotton species similar to cultivated cotton exist, EPA has prohibited the sale or distribution of Bt cotton in those areas, on the premise that these containment measures would prevent the movement of the registered Bt endotoxin from Bt cotton to wild or feral relatives (US-EPA, 2008a).

Feral populations of *G. hirsutum* have been listed as threatened and endangered by the State of Florida (Coile and Garland, 2003). However, in Florida, wild *G. hirsutum* is not present in the northwestern panhandle where cotton cultivation occurs (Coile and Garland, 2003; USDA-NASS, 2007; Wunderlin and Hansen, 2010).

Horizontal gene transfer and consequent expression of DNA from a plant species to bacteria is unlikely to occur (Keese, 2008). Many bacteria (or parts thereof) that are closely associated with plants have been sequenced, including *Agrobacterium* and *Rhizobium* (Kaneko et al., 2000; Kaneko et al., 2002; Wood et al., 2001). There is no evidence that these organisms contain genes derived from plants. In cases where the review of sequence data implied that horizontal gene transfer occurred, these events were inferred to occur on an evolutionary time scale in the order of millions of years (Brown, 2003; Koonin et al., 2001).

2.5 PUBLIC HEALTH

Public health concerns surrounding GE cotton, such as TwinLink™ Cotton, focus primarily on human and animal consumption. Although cotton is grown primarily for textile production, valuable food oil is produced from cotton seed. The remaining seed residue (cottonseed meal and hulls) is used as livestock feed and as a high-protein flour (OECD, 2009). Cotton seed meal animal feed provides protein, fat, and energy (National Cottonseed Products Association, 2002).

Non-GE cotton varieties, both those developed for conventional use and for use in organic production systems, are not routinely required to be evaluated by any regulatory agency in the U.S. for human food safety prior to release in the market. Under the FFDCA, it is the responsibility of food manufacturers to ensure that the products they market are safe and properly labeled. Food derived from TwinLink™ Cotton must be in compliance with all applicable legal and regulatory requirements. GE organisms for food and feed may undergo a voluntary consultation process with the FDA prior to release onto the market. Although a voluntary process, thus far, all applicants who have wished to commercialize a GE variety that will be included in the food supply have completed a consultation with the FDA. In a consultation, a developer who intends to commercialize a bioengineered food meets with the agency to identify and discuss relevant safety, nutritional, or other regulatory issues regarding the bioengineered food and then submits to FDA a summary of its scientific and regulatory assessment of the food. FDA evaluates the submission and responds to the developer by letter (US-FDA, 2010).

Bayer has provided the FDA with information on the identity, function, and characterization of the genes for TwinLink™ Cotton, including expression of the gene products. The FDA is currently reviewing the information submitted by the applicant. In the absence of a completed FDA determination, APHIS takes into consideration prior FDA reviews of comparable products to make a preliminary assessment of the potential food and safety impacts. Note that with regard to the expression of glufosinate ammonium tolerance, the gene construct in the Bayer TwinLink™ Cotton is the same as that approved by the FDA in June 2003 for the LibertyLink® Cotton product (US-FDA, 2003). In that approval, the FDA noted that the transformational event in LibertyLink® Cotton was not materially different in composition, safety, or any other relevant parameter than other cotton grown, marketed and consumed at that time (US-FDA, 2003). This previous FDA review will be used by APHIS to analyze the food and safety impacts associated with the incorporation and expression of glufosinate ammonium tolerance in TwinLink™ Cotton.

The FDA's oversight of the food and safety impacts associated with the incorporation and expression of pesticidal substances, in this case, the Cry proteins associated with Bt, are more limited. The EPA is the primary authority for the review of plant-incorporated protectants.

EPA Section 3 FIFRA registration applications have been submitted by Bayer to the EPA for Cry1Ab cotton, Cry2Ae cotton and TwinLink™ Cotton. EPA is currently reviewing information submitted by the applicant on the efficacy and potential environmental concerns associated with the use of this product. APHIS has been advised that the product will need three separate new active ingredient registrations under Section 3 of FIFRA:

- One for Cry1Ab from event 3T04-40.
- One for Cry2Ae from event GHB119.
- One for the stacked event combining both Cry proteins.

In the absence of a completed EPA determination, APHIS takes into consideration prior EPA reviews of comparable products to make a preliminary assessment of the potential impacts. The EPA, in 2008, published a Biopesticides Registration Action Document (BRAD) for *Bacillus thuringiensis* (*Bt*) incorporated protectants (US-EPA, 2008a). Based on a comprehensive human health effects assessment, the Bt BRAD found no unreasonable adverse effects associated with exposure to the Bt products. The Bt protein is a registered microbial pesticide which is approved for use on food crops. The EPA concluded in the BRAD that the Bt products incorporated as plant protectants would be expected to behave as expected of a dietary protein (US-EPA, 2008a). A tolerance exemption is in place for Cry1Ab protein in all crops (40 CFR §174.511; US-EPA, 2010d), as well as for the PAT protein (40 CFR §174.522; US-EPA, 2010g). A temporary exemption from the requirement of a tolerance has been issued for Cry2Ae (40 CFR §174.530; US-EPA, 2010e). This previous EPA review will be used by APHIS to analyze the food and safety impacts associated with the incorporation and expression of Cry proteins in TwinLink™ Cotton.

Glufosinate ammonium is currently registered under various trade names for control of weeds in cotton and other crops; the Ignite®/Liberty® trade name is registered for use on LibertyLink® corn, cotton, soy, rice and canola varieties (BCS, 2010a; Scott, 2008). EPA's core pesticide risk assessment and regulatory processes ensure that protections are in place for all populations of nontarget species. These assessments provide EPA with information needed to develop label use restrictions for the pesticide. Growers are required to use pesticides, such as glufosinate ammonium, consistent with the application instructions provided on the EPA-approved pesticide label (see, e.g., BCS, 2010a). These label restrictions carry the weight of law and are enforced by EPA and the states (Federal Insecticide, Fungicide, and Rodenticide Act 7 USC 136j (a)(2)(G) Unlawful Acts). Therefore, it is expected that glufosinate ammonium use on the TwinLink™ Cotton product would be consistent with the EPA-approved label.

Glufosinate ammonium acts in plants by inhibiting the enzyme glutamine synthase causing a toxic buildup of ammonia within the treated plant (Scott, 2008). Glufosinate ammonium is a nonselective herbicide for both non-crop and crop uses. It is highly biodegradable, has no residual activity, and has very low toxicity for humans. Glufosinate ammonium degrades rapidly in microbially active soils and also readily binds to soil particles, and its short-lived metabolites have not been found to accumulate in the environment. Soil microorganisms, bees, earthworms, birds, and mammals are unaffected by glufosinate ammonium (Scott, 2008). A food tolerance for residues of glufosinate ammonium has been published (40 CFR §180.473; US-EPA, 2010c).

2.6 ANIMAL FEED

As noted in the discussion of Public Health (Subsection 2.5), seed residue remaining after fiber removal for textile production (cottonseed meal and hulls) is marketed as cottonseed meal, cottonseed hulls, and whole cottonseed and utilized in the animal feed industry as sources of protein, fiber and energy (National Cottonseed Products Association, 2002; OECD, 2009). The value of cottonseed as animal feed represents a substantial portion of the grower's income from cotton (Blasi and Drouillard, 2002).

Cottonseed meal is usually sold as a high protein animal feed supplement, providing as much as 41% protein by weight (National Cottonseed Products Association, 2002). The meal can be used alone or in combination with other plant and animal protein sources (National Cottonseed Products Association, 2002). Cottonseed hulls are the outer covering of the cotton seed which are removed prior to oil extraction (National Cottonseed Products Association, 2002). The hulls provide a source of fiber to animal feed rations (National Cottonseed Products Association, 2002). Whole cottonseed is the seed remaining after the long fibers have been removed (National Cottonseed Products Association, 2002). The whole cotton seed contains a small quantity of attached linters, and as animal feed provides protein, energy and fiber (National Cottonseed Products Association, 2002).

Although cottonseed meal is a widely used animal feed (Blasi and Drouillard, 2002; Calhoun, 2011), gossypol, one of the chemical constituents of cottonseed meal, is potentially toxic (Gadberry, 2011). Ruminants (i.e. cattle) have the ability to detoxify gossypol during the fermentation process in the gut (Gadberry, 2011). Gossypol has been identified as potentially causing reduced fertility in young developing bulls, resulting in recommendations to limit cottonseed meal in the diet for developing bulls (Blasi and Drouillard, 2002; University of Arkansas Cooperative Extension Service, 2011).

Non-GE cotton varieties, both those developed for conventional use and for use in organic production systems, are not routinely required to be evaluated by any regulatory agency in the U.S. for animal feed safety prior to release in the market. Under the FFDCA, it is the responsibility of food and feed manufacturers to ensure that the products they market are safe and properly labeled. Feed derived from TwinLink™ Cotton must be in compliance with all applicable legal and regulatory requirements. GE organisms for feed may undergo a voluntary consultation process with the FDA prior to release onto the market. Although a voluntary process, thus far, all applicants who have wished to commercialize a GE variety that will be included in the food supply have completed a consultation with the FDA. In a consultation, a developer who intends to commercialize a bioengineered food meets with the agency to identify and discuss relevant safety, nutritional, or other regulatory issues regarding the bioengineered food and then submits to FDA a summary of its scientific and regulatory assessment of the food. FDA evaluates the submission and responds to the developer by letter (US-FDA, 2010).

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2.7 SOCIOECONOMIC ISSUES

Cotton has been cultivated for fiber for over 5,000 years (OECD, 2008). Cotton is grown primarily for its lint fibers which are made into textiles (OECD, 2009). Cotton seed also produces valuable food oil and the remaining seed residue (cottonseed meal and hulls) is used as livestock feed and as a high-protein flour (OECD, 2009). The remainder of the plant is left in the field for decomposition as fertilizer (OECD, 2009).

In the U.S. for the 2010 production year, cotton was grown on 4.4 million hectares (10.9 million acres (USDA-NASS, 2010b). Cotton is produced in the Cotton Belt, 17 southern states, from Virginia to California (Meyer et al., 2007; USDA-NASS, 2010c). The major cotton producing states are Texas (5.4 million acres), Georgia (1.3 million acres), Arkansas (0.540 million acres), Mississippi (0.420 million acres), North Carolina (0.545 million acres) and Tennessee (0.387 million acres) (USDA-NASS, 2010c). Texas, Arkansas, Georgia, North Carolina, and Mississippi collectively produced over 58% of the cotton in 2010 (USDA-NASS, 2010e). In 2007, total cotton cultivated land in the U.S. was about 10,500,000 acres (slightly larger than the combined size of the states of Maryland and Connecticut) with a market value of about $5 billion (USDA-NASS, 2007).

Cotton acreage in the U.S. rose slightly during the first half of the 2000s, with a corresponding increase in production (USDA-ERS, 2009). The increase in production has been attributed to advances in technology (seed varieties, fertilizers, pesticides, and machinery) and production practices (reduced tillage, irrigation, crop rotations, and pest management systems) (USDA-ERS, 2009). The impact of these changes has been particularly evident, with yields and production reaching new highs (USDA-ERS, 2009). Since 2006, however, U.S. cotton planted area has been considerably lower, as relative prices have favored the planting of alternative crops, such as corn and soybeans (USDA-ERS, 2009). All regions of the Cotton Belt have experienced significant declines compared with the first half of the 2000s (USDA-ERS, 2009). Although U.S. cotton production decreased considerably following the area reductions of the late 2000s, consistently higher yields helped limit the effect of these acreage declines (USDA-ERS, 2009).

According to the Census of Agriculture, U.S. cotton farms numbered 18,605 in 2007, down from 24,805 in 2002 (USDA-ERS, 2009). Although the number has fallen, cotton acreage per farm has risen, averaging 564 acres per farm in 2007 compared with 502 acres in 2002. The percentage of large cotton farms (over 1,000 acres) has continued to increase, but the share of small cotton farms (under 100 acres) has declined (USDA-ERS, 2009).

The U.S. cotton industry generates about 200,000 jobs among the various sectors from farm to textile mill, and accounts for more than $25 billion in products and services annually (USDA-ERS, 2009).

Total 2010 cotton production is forecast at 18.9 million 480-pound bales, up 55% from 2009 production of 12.2 million bales (USDA-NASS, 2010e). Yield is expected to average 841 pounds per harvested acre, up 64 pounds from 2009 (USDA-NASS, 2010e). Upland cotton production is forecast at 18.4 million 480-pound bales, and 56% above 2009 (USDA-NASS, 2010e). American Pima production is forecast at 497,800 bales (USDA-NASS, 2010e).

Trade is particularly important for cotton. About 30% of the world's consumption of cotton fiber crosses international borders before processing, a larger share than for wheat, corn, soybeans, or rice (USDA-ERS, 2009). Through trade in yarn, fabric, and clothing, much of the world's cotton again crosses international borders at least once more before reaching the final consumer (USDA-ERS, 2009).

Consumption of cotton by U.S. textile mills peaked in 1997; since then, U.S. mill use of cotton has plummeted, dropping about 50% by 2005 and nearly 70% by 2009 (USDA-ERS, 2009). Although the end of the Multifibre Arrangement's (MFA in 2005 was a factor, much of the decline in U.S. textile production occurred before then. The MFA was a multilateral agreement signed in 1974, but it began in the 1930s as a mechanism to protect domestic textile markets, establishing strict limits on imports from Japan, Hong Kong, Pakistan and India, among many others (Meyer et al., 2007). Under the MFA, Canada, the U.S., and the EU could set limits on the amount of foreign-made apparel and textiles they would allow into their countries (Meyer et al., 2007). The MFA ended after the 1995 Uruguay Round Agreements on trade liberalization (Meyer et al., 2007). The result of this change is increased global competition for import and export of raw cotton, as well as finished textiles (Meyer et al., 2007). U.S. consumer demand for cotton products remains strong, but imported clothing now accounts for most purchases by U.S. consumers (USDA-ERS, 2006). The changes after the end of the MFA is reflected in the importance of exports for cotton growers. The USDA ERS reports that U.S. cotton mills consumed 60% of the domestic cotton through the 1990s, but not long after the ending of the MFA quotas, 70% of the U.S. cotton was exported (Meyer et al., 2007).

The cotton industry continues to face many of the supply and demand concerns confronting other field crops. However, because cotton is used primarily in manufactured products, such as clothing and home furnishings, the industry faces additional challenges associated with the economic well-being of downstream manufacturing industries, as well as the economic well-being of the final consumer (USDA-ERS, 2009).

The USDA ERS notes that U.S. cotton productivity has increased in recent years as a result of technological advancements, including biotechnology-derived varieties (Meyer et al., 2007). The introduction of cotton providing insect resistance or herbicide tolerance also has global implications. Herbicide-tolerant cotton is one of the most widely and rapidly adopted crops in the U.S., followed by insect-resistant cotton (USDA-ERS, 2010a). In 2010, GE varieties of cotton were cultivated on 93% of the acreage in the U.S., with herbicide-tolerant cotton cultivated on 78% of the acreage, and Bt cotton cultivated on 73% of the acreage (USDA-ERS, 2010a).

Outside the U.S., Bt cotton has been widely adopted in India and China, two major cotton producers (Meyer et al., 2007). The cost savings of Bt cotton brought millions of hectares back into cotton production in eastern China, and has also helped India's cotton area rebound by more than 1 million hectares (Meyer et al., 2007). Bt cotton has also been adopted in smaller producing countries like Australia, Argentina, Mexico, and South Africa (Meyer et al., 2007).

3 ALTERNATIVES

This document analyzes the potential environmental consequences of a determination of nonregulated status of BCS TwinLink™ Cotton. To respond favorably to a petition for nonregulated status, APHIS must determine that TwinLink™ Cotton is unlikely to pose a plant pest risk. Based on its Plant Pest Risk Assessment (USDA-APHIS, 2010b) APHIS has concluded that TwinLink™ Cotton is unlikely to pose a plant pest risk. Therefore, APHIS must determine that COT67B cotton is no longer subject to 7 CFR part 340 or the plant pest provisions of the Plant Protection Act.

Two alternatives will be evaluated in this EA: 1) no action; and 2) determination of nonregulated status of TwinLink™ Cotton . APHIS has assessed the potential for environmental impacts for each alternative in Section 4 of this EA, Environmental Consequences.

3.1 NO ACTION: CONTINUATION AS A REGULATED ARTICLE

Under the No Action Alternative, APHIS would deny the petition. TwinLink™ Cotton and progeny derived from TwinLink™ Cotton would continue to be regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of TwinLink™ Cotton and measures to ensure physical and reproductive confinement would continue to be implemented. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of TwinLink™ Cotton.

This alternative is not the Preferred Alternative because APHIS has concluded through a Plant Pest Risk Assessment (USDA-APHIS, 2010b) that TwinLink™ Cotton is unlikely to pose a plant pest risk. Choosing this alternative would not satisfy the purpose and need of making a determination of plant pest risk status and responding to the petition for nonregulated status.

3.2 PREFERRED ALTERNATIVE: DETERMINATION THAT TWINLINK™ COTTON IS NO LONGER A REGULATED ARTICLE

Under this alternative, TwinLink™ Cotton and progeny derived from them would no longer be regulated articles under the regulations at 7 CFR Part 340. TwinLink™ Cotton is unlikely to pose a plant pest risk (USDA-APHIS, 2010b). Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of TwinLink™ Cotton and progeny derived from this event. This alternative best meets the agency's purpose and need to respond appropriately to a petition for nonregulated status based on the requirements in 7 CFR part 340 and the agency's authority under the plant pest provisions of the Plant Protection Act. Because the agency has concluded that TwinLink™ Cotton is unlikely to pose a plant pest risk, a determination of nonregulated status of TwinLink™ Cotton is a response that is consistent with the plant pest provisions of the PPA, the regulations codified in 7 CFR part 340, and the biotechnology regulatory policies in the Coordinated Framework.

Under this alternative, growers may have future access to TwinLink™ Cotton and progeny derived from this event if the developer decides to commercialize TwinLink™ Cotton.

3.3 ALTERNATIVES CONSIDERED BUT REJECTED FROM FURTHER CONSIDERATION

APHIS assembled a list of alternatives that might be considered for TwinLink™ Cotton. The agency evaluated these alternatives, in light of the agency's authority under the plant pest provisions of the Plant Protection Act, and the regulations at 7 CFR part 340, with respect to environmental safety, efficacy, and practicality to identify which alternatives would be further considered for TwinLink™ Cotton. Based on this evaluation, APHIS rejected several alternatives. These alternatives are discussed briefly below along with the specific reasons for rejecting each.

3.3.1 Prohibit Any TwinLink™ Cotton from Being Released

In response to public comments that stated a preference that no GE organisms enter the marketplace, APHIS considered prohibiting the release of TwinLink™ Cotton, including denying any permits associated with the field testing. APHIS determined that this alternative is not appropriate given that APHIS has concluded that TwinLink™ Cotton is unlikely to pose a plant pest risk (USDA-APHIS, 2010b).

In enacting the Plant Protection Act, Congress found that

> [D]ecisions affecting imports, exports, and interstate movement of products regulated under [the Plant Protection Act] shall be based on sound science… § 402(4).

On March 11, 2011, in a Memorandum for the Heads of Executive Departments and Agencies, the White House Emerging Technologies Interagency Policy Coordination Committee developed broad principles, consistent with Executive Order 13563, to guide the development and implementation of policies for oversight of emerging technologies (such as genetic engineering) at the agency level. In accordance with this memorandum, agencies should adhere to Executive Order 13563 and, consistent with that Executive Order, the following principle, among others, to the extent permitted by law, when regulating emerging technologies:

> “[D]ecisions should be based on the best reasonably obtainable scientific, technical, economic, and other information, within the boundaries of the authorities and mandates of each agency”

Based on our Plant Pest Risk Assessment (USDA-APHIS, 2010b) and the scientific data evaluated therein, APHIS has concluded that TwinLink™ Cotton is unlikely to pose a plant pest risk. Accordingly, there is no basis in science for prohibiting the release of TwinLink™ Cotton.

3.3.2 Approve the petition in part

The regulations at 7 CFR 340.6(d)(3)(i) state that APHIS may "approve the petition in whole or in part." For example, a determination of nonregulated status in part may be appropriate if there is a plant pest risk associated with some, but not all lines described in a petition. Because APHIS has concluded that TwinLink™ Cotton is unlikely to pose a plant pest risk, there is no regulatory basis under the plant pest provisions of the Plant Protection Act for considering approval of the petition only in part.

3.3.3 Isolation distance between TwinLink™ Cotton and non-GE cotton and geographical restrictions

In response to public concerns of gene movement between GE and non-GE plants, APHIS considered requiring an isolation distance separating TwinLink™ Cotton from non-GE cotton production. However, because APHIS has concluded that TwinLink™ Cotton is unlikely to pose a plant pest risk (USDA-APHIS, 2010b), an alternative based on requiring isolation distances would be inconsistent with the statutory authority under the plant pest provisions of the Plant Protection Act and regulations in 7 CFR part 340.

APHIS also considered geographically restricting the production of TwinLink™ Cotton based on the location of production of non-GE cotton in organic production systems in response to public concerns regarding possible gene movement between GE and non-GE plants. However, as presented in APHIS' plant pest risk assessment for TwinLink™ Cotton, there are no geographic differences associated with any identifiable plant pest risks for TwinLink™ Cotton (USDA-APHIS, 2010b). This alternative was rejected and not analyzed in detail because APHIS has concluded that TwinLink™ Cotton does not pose a plant pest risk, and will not exhibit a greater plant pest risk in any geographically restricted area. Therefore, such an alternative would not be consistent with APHIS' statutory authority under the plant pest provisions of the Plant Protection Act and regulations in Part 340 and the biotechnology regulatory policies embodied in the Coordinated Framework.

Based on the foregoing, the imposition of isolation distances or geographic restrictions would not meet APHIS' purpose and need to respond appropriately to a petition for nonregulated status based on the requirements in 7 CFR part 340 and the agency's authority under the plant pest provisions of the Plant Protection Act. Nevertheless, APHIS is not expecting significant effects. However, individuals might choose on their own to geographically isolate their non-GE cotton productions systems from TwinLink™ Cotton or to use isolation distances and other management practices to minimize gene movement between cotton fields.

3.3.4 Requirement of Testing For TwinLink™ Cotton

During the comment periods for other petitions for nonregulated status, some commenters requested USDA to require and provide testing to identify GE products in non-GE production systems. APHIS notes there are no nationally-established regulations involving testing, criteria, or limits of GE material in non-GE systems. Such a requirement would be extremely difficult to implement and maintain. Additionally, because TwinLink™ Cotton does not pose a plant pest risk (USDA-APHIS, 2010b), the imposition of any type of testing requirements is inconsistent with the plant pest provisions of the Plant Protection Act, the regulations at 7 CFR part 340 and the biotechnology regulatory policies embodied in the Coordinated Framework. Therefore, imposing such a requirement for TwinLink™ Cotton would not meet APHIS' purpose and need to respond appropriately to the petition in accordance with its regulatory authorities.

3.4 COMPARISON OF ALTERNATIVES

Table 3-1 presents a summary of the potential impacts associated with selection of either of the alternatives evaluated in this EA. The impact assessment is presented in Section 4 of this EA.

Table 3-1: Summary of Potential Impacts and Consequences of Alternatives

Attribute/Measure	**Alternative A: No Action**	**Alternative B: Determination of Nonregulated Status**
Meets Purpose and Need and Objectives	**No**	**Yes**
Unlikely to pose a plant pest risk	Satisfied through use of regulated field trials	Satisfied – risk assessment (USDA-APHIS, 2010b)
Management Practices		
Acreage and Areas of Cotton Production	Unchanged	Unchanged
Cropping Practices	Unchanged	Unchanged
Seed Production	Unchanged	Unchanged
Organic Farming	Unchanged	Unchanged
Specialty Cotton Production	Unchanged	Unchanged
Physical Environment		
Water Resources	Unchanged	Unchanged
Soil	Unchanged	Unchanged
Air Quality	Unchanged	Unchanged
Climate Change	Unchanged	Unchanged
Animal and Plant Communication		
Animals	Unchanged	Unchanged
Plants	Unchanged	Minimal
Biological Diversity	Unchanged	Minimal
Gene Movement	Unchanged	Unchanged
Public Health		
Human Health	Unchanged	Unchanged
Worker Safety	Unchanged	Unchanged
Animal Feed	Unchanged	Unchanged
Socioeconomic Issues		
Domestic Economic Environment	Unchanged	Unchanged
Trade Economic Environment	Unchanged	Unchanged
Social Environment	Unchanged	Unchanged
Other Cumulative Effects	Unchanged	Unchanged
Threatened and Endangered Species	Unchanged	Unchanged
Other U.S Regulatory Approvals	Unchanged for existing deregulated GE organisms	FDA consultation pending, EPA tolerance exemptions and conditional pesticide registrations being reviewed
Compliance with Other Laws		
CWW, CAA, EOs	Fully compliant	Fully compliant

Notes:

1. Unchanged – the current conditions will not change as a result of the selection of this alternative.
2. Minimal – the current conditions may change slightly as a result of the selection of this alternative, but the changes, if any, are not deemed significant.

4 ENVIRONMENTAL CONSEQUENCES

This analysis of potential environmental consequences addresses the potential impact to the human environment from the alternatives analyzed in this EA, namely taking no action and a determination by the agency that TwinLink™ Cotton does not pose a plant pest risk. Potential environmental impacts from the No Action Alternative and the Preferred Alternative for TwinLink™ Cotton are described in detail throughout this section. A cumulative effects analysis is also included for each environmental issue and includes a discussion of the cumulative impacts that are associated with the Preferred Alternative, when combined with other recent past, present and reasonably foreseeable future actions within the affected environment. Certain aspects of this product and its cultivation would be no different between the alternatives; those instances are described below.

4.1 SCOPE OF THE ENVIRONMENTAL ANALYSIS

For the discussion of environmental consequences, this section addresses the following principal areas of potential environmental concern:

- Agricultural Production of Cotton (Subsection 4.2);
- Physical Environment (Subsection 4.3);
- Animal and Plant Communities (Subsection 4.4);
- Public Health (Subsection 4.5);
- Animal Feed (Subsection 4.6);
- Socioeconomic Issues (Subsection 4.7);
- Other Cumulative Effects (Subsection 4.8);
- Threatened and Endangered Species (Subsection 4.9); and
- Consideration of Executive Orders, Standards, and Treaties Relating to Environmental Impacts (Subsection 4.10).

Although the Preferred Alternative would allow for new plantings of TwinLink™ Cotton to occur anywhere in the U.S., APHIS is limiting the environmental analysis to those areas that currently support cotton production, as identified by the National Agricultural Statistics Service (NASS) 2007 Census of Agriculture. The NASS found that cotton is currently produced in the following 17 states: Alabama, Arizona, Arkansas, California, Florida, Georgia, Kansas, Louisiana, Mississippi, Missouri, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia (USDA-NASS, 2010c).

The environmental consequences of the No Action and Preferred Alternatives are analyzed under the assumption that farmers who produce conventional cotton, TwinLink™ Cotton, or cotton using organic methods are using reasonable, commonly accepted best management practices specific to their agricultural cotton production. However, APHIS recognizes that not all farmers follow these best management practices for cotton. Thus, the analyses of the environmental effects will also include the assumption that some farmers do not follow these best management practices.

4.2 AGRICULTURAL PRODUCTION OF COTTON

One of APHIS's missions is to improve American agricultural productivity. Best management practices are commonly accepted, practical ways to grow cotton, regardless of whether the cotton farmer is using conventional practices with non-GE or GE varieties, or organic practices. These management practices consider crop-specific planting dates, seeding rates, and harvest times, among others. Over the years, cotton production has resulted in well-established, widely-practiced management practices that are available through local Cooperative Extension Service offices and their respective websites. The National Information System for the Regional Integrated Pest Management Centers publishes crop profiles for major crops on a state-by-state basis. These crop profiles provide production guidance for local growers, including recommended practices for specific pest control. Crop profiles for many of the cotton production states can be reviewed at (www.ipmcenters.org/cropprofiles/index.cfm).

BCS' studies demonstrate that agronomic characteristics and cultivation practices required for TwinLink™ Cotton are essentially indistinguishable from other cotton varieties, including other herbicide-tolerant and insect-resistant varieties (Scott, 2008; USDA-APHIS, 2010b). Although TwinLink™ Cotton might be expected to replace other varieties of cotton currently cultivated, new acreage is not expected to be developed to accommodate the cultivation of TwinLink™ Cotton (Scott, 2008). None of the best management practices currently employed for cotton production is expected to change if TwinLink™ Cotton is deregulated. Accordingly, the potential impacts on agricultural production of TwinLink™ Cotton resulting from management practices associated with the No Action and Preferred Alternatives are the same.

4.2.1 Acreage and Areas of Cotton Production

GE and non-GE cotton varieties are continually under development. Cotton acreage averaged over 14.5 million acres during the first half of the 2000s (USDA-APHIS, 2010a). Since 2006, however, U.S. cotton planted acreage has been considerably lower as market prices have favored the planting of alternative crops, such as corn and soybeans (USDA-APHIS, 2010a). Most of the U.S. cotton acreage is planted in GE cotton. Of the 11.3 million acres planted in cotton in 2010, 93% (10.5 million acres) were GE cotton. Of this, 73% of the GE cotton acreage was GE insect-resistant (Bt) cotton and 78% was herbicide-tolerant (USDA-ERS, 2010a, 2010c). Most cotton is planted on farms that have been in cotton production for at least five years (USDA-NASS, 2007).

<u>No Action: Acreage and Areas of Cotton Production</u>

Based on current acreage trends, conventional cotton production practices with GE varieties will likely continue to dominate or perhaps increase in acreage under the No Action Alternative. Cotton is currently produced commercially in 17 states (USDA-NASS, 2010c) and under the No Action Alternative, this range of production is not expected to change.

<u>Preferred Alternative: Acreage and Areas of Cotton Production</u>

BCS' studies demonstrate that agronomic characteristics and cultivation practices required for TwinLink™ Cotton are essentially indistinguishable from other cotton varieties, including other herbicide-tolerant and insect-resistant varieties (Scott, 2008; USDA-APHIS, 2010b). BCS posits that although TwinLink™ Cotton might be expected to replace other varieties of cotton currently cultivated, new acreage is not expected to be developed to accommodate the cultivation of TwinLink™ Cotton (Scott, 2008). The introduced lepidopteran resistance trait and the herbicide tolerance trait do not confer any competitive advantage in terms of weediness or to extend the range of cultivation outside of existing cultivation areas (Scott, 2008). The Preferred Alternative, a determination of nonregulated status of TwinLink™ Cotton, is thus not expected to increase cotton production, either by its availability alone or accompanied by other factors, or cause an increase in overall GE cotton acreage. Impacts would be similar to the No Action Alternative.

Cumulative Effects: Acreage and Areas of Cotton Production

Cumulative effects of a determination of nonregulated status of TwinLink™ Cotton are unlikely. Neither the No Action Alternative nor a determination of nonregulated status of TwinLink™ Cotton is expected to directly cause an increase in agricultural acreage devoted to cotton production or those cotton acres devoted to GE cotton cultivation. The availability of TwinLink™ Cotton will not change cultivation areas for cotton production in the U.S. and there are no anticipated changes to the availability of GE and non-GE cotton varieties on the market under either alternative.

4.2.2 Cropping Practices: Crop Rotation and Pesticide Use

Crop rotation in cotton is conducted to optimize soil nutrition and fertility, reduce pathogen loads, and control certain cotton pests (Gazaway et al., 2007); (University of California IPM Online, 2008a). Crop rotation practices have been described previously in Section 2. Crop rotation practices are not expected to change with the planting and production of TwinLink™ Cotton.

In the U.S., the cotton industry has consistently relied heavily on insecticide use strategies to manage arthropod pests (Gianessi and Carpenter, 1999). Insecticides were applied to 66% of the cotton acreage in 2007 (USDA-NASS, 2008). Of thirty listed insecticides, Acephate was the most utilized insecticide, with 26% of the planted acreage being treated at an average rate of 0.900 pounds per acre per crop year (USDA-NASS, 2008). Dicrotophos was the second most commonly utilized insecticide, applied to 21% of the acreage at an average rate of 0.565 pounds per year (USDA-NASS, 2008). Other insecticides applied in the 2007 growing year included Acetamiprid (6% of acreage), Cyfluthrin (8% of acreage), Cypermethrin (7% of acreage), Imadacloprid (6% of acreage), Lambda-cyhalothrin (5% of acreage), Malathion (5% of acreage), and Thiamethoxam (11% of acreage).

Resistance to commonly used insecticides (pyrethroids, organophosphates, and carbamates) has led to a need for new insecticide chemistries or other novel approaches such as the use of sterile insects, pathogens, and transgenic cotton (Gianessi and Carpenter, 1999). Cotton varieties expressing the Bt endotoxin have emerged as a viable product replacing many of these commonly used pesticides (Scott, 2008).

One of the documented benefits of Bt cotton has been the reduction in the use of broad-spectrum insecticides during cotton production (Benbrook, 2009; Fernandez-Cornejo and Caswell, 2006).

Each acre planted in Bt cotton is presumed to displace the average pounds of insecticide previously sprayed on an acre of conventional cotton for lepidopteran control (Benbrook, 2009). In a comprehensive evaluation of 13 years of USDA NASS data (1996 through 2008) on pesticide usage (including both herbicides and insecticides), Benbrook found that pesticide application rates for managing lepidopteran pests in cotton changed in direct proportion to the adoption of Bt Cotton. Table 4-1 provides an excerpt from Benbrook's summary of insecticide use.

Table 4-1: Summary of Insecticide Use for Lepidopteran Control in Cotton Compared with Adoption of Bt Cotton*

Cotton Crop Type	% Acreage by Year			
	1996	2001	2005	2008
Planted with Bt Cotton	12%	41%	60%	73%
Treated with Insecticides	48%	31%	30%	25%

*Benbrook's study reports all 13 years of data. This table presents an extracted summary to show use trends.

Source: Benbrook (2009) Supplemental Table 12. Insecticides Applied to Control the Budworm/Bollworm Complex of Insects on Conventional Varieties of Cotton, 1996 – 2008.

Benbrook's assessment presents strong evidence that there has been a dramatic reduction in the use of insecticides to control lepidopteran pests in cotton directly associated with the adoption of Bt cotton.

Cotton cultivation also requires substantial use of herbicides for weed control during the growing season and for chemical defoliation prior to harvest (Scott, 2008). In 2007, 35 different herbicides were applied to 97% of the cotton acreage (USDA-NASS, 2008). Glyphosate isopropylamine salt was the most commonly applied herbicide, applied to 85% of the planted acres at a rate of 1.900 pounds per acre per crop year (USDA-NASS, 2008). The next two most commonly applied herbicides on a per acre basis were Trifluralin and Diuron, at 29 and 26% with average application rates of 0.921 and 0.499 pounds per acre per crop year, respectively (USDA-NASS, 2008). Other herbicides applied to cotton included 2,4-D (6% of the acreage), Carfentrazone-ethyl (10% of the acreage), Flumioxazin (6% of the acreage), Pendimethalin (17% of the acreage), Prometryn (7% of the acreage), Pyraflufen-ethyl (8% of the acreage), pyrithiobac-sodium (10% of the acreage), and S-metolachlor (6% of the acreage) (USDA-NASS, 2008).

The consequences of adoption of herbicide tolerance show a contrasting trend. Herbicide-tolerant cotton was first grown commercially in the U.S. in 1997 and by 2008, herbicide-tolerant cotton was planted on 68% of total cotton plantings (Brookes and Barfoot, 2010). Stacked varieties, providing both herbicide tolerance and insect resistance, comprised nearly 93% of the total plantings in 2008 (Benbrook, 2009). Benbrook has reported that the adoption of herbicide-tolerant crops has resulted in an increase in the volume of herbicides applied to crops (Benbrook, 2009). Benbrook notes that herbicide use declined between 1996 and 2001, apparently in direct response to the adoption of herbicide-tolerant cotton (Benbrook, 2009). However, since that time, herbicide use has increased. Reported increases in herbicide use reflect an increase in use of conventional herbicides, as well as an increase in glyphosate applications (Benbrook, 2009). Benbrook concludes that this reported increase in herbicide use is a direct consequence of the development of glyphosate-resistant weeds in cotton. As of 2008, nine weeds were identified as glyphosate-resistant, with three of these weeds, horseweed (marestail), Johnsongrass, and pigweed (Palmer amaranth) specifically identified as difficult to control weeds in cotton.

No Action: Cropping Practices: Crop Rotation and Pesticide Use

Under the No Action Alternative, cotton crop rotation practices and pesticide use is expected to remain as practiced today by the farming community. Growers will continue to have access to existing deregulated GE lepidopteran-resistant and herbicide-tolerant cotton varieties, as well as conventional cotton varieties. It is likely that growers would continue to experience the continued emergence of glyphosate-tolerant weeds, requiring modifications of crop management practices to address these weeds. These changes may involve all of the techniques identified by Benbrook (2009), including use of other herbicides for weed control.

Preferred Alternative: Cropping Practices: Crop Rotation and Pesticide Use

BCS' studies demonstrate TwinLink™ Cotton is essentially indistinguishable from other cotton varieties used in terms of agronomic characteristics and cultivation practices (Scott, 2008; USDA-APHIS, 2010b). Therefore, cotton crop rotation practices are expected to be unchanged under the Preferred Alternative.

If TwinLink™ Cotton is adopted, a continued reduction in the use of budworm/bollworm insecticides applications and the number of acre-treatments per year as reported in Benbrook's trend analysis is expected to occur. The deregulation of TwinLink™ Cotton will provide growers with another alternative Bt cotton variety to cultivate. Herbicide use patterns have the potential to change as well. The introduction of TwinLink™ Cotton provides a stacked variety, expressing Bt-based lepidopteran resistance combined with tolerance to glufosinate ammonium, an alternative herbicide. TwinLink™ Cotton provides growers with an alternative to those cotton varieties resistant to glyphosate, thus expanding options in the field for weed control.

In those fields where glyphosate-resistant weeds have emerged, glufosinate ammonium tolerance provides the grower with an option to transition away from glyphosate herbicides to a different post-emergent herbicide. The transition to glufosinate ammonium could reduce applications of those other herbicides needed to manage glyphosate-resistant weeds.

Cumulative Effects: Cropping Practices: Crop Rotation and Pesticide Use

A determination of nonregulated status of TwinLink™ Cotton is not expected to result in changes in the current cotton cropping practices. APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to affect changes in crop rotation. Studies demonstrate TwinLink™ Cotton is essentially indistinguishable from other cotton varieties used in terms of agronomic characteristics and cultivation practices (Scott, 2008).

It is anticipated that the trend of reduced broad-spectrum insecticide use by cotton growers will continue due to the adoption of Bt cotton and other cultural practices. There is the potential that the introduction of glufosinate ammonium tolerance may result in a reduction in total herbicide use as growers adopt different herbicide treatment strategies involving glufosinate ammonium as a post-emergent crop treatment, thereby reducing the use of some of the other herbicides which have been required in response to the emergence of glyphosate-resistant weeds.

4.2.3 Seed Production

As discussed in Subsection 2.2.3, cotton seed production is managed through the AOSCA standard procedures to preclude gene flow between species and varieties (Wozniak, 2002). Common practices include: 1) maintaining isolation distances to prevent pollen movement from other cotton sources; 2) planting border or barrier rows to intercept pollen; 3) employing natural barriers to pollen; and 4) field monitoring for off-types, other crops, weeds, and disease.

<u>No Action: Seed Production</u>

Under the No Action Alternative, current cotton seed production practices are not expected to change.

<u>Preferred Alternative: Seed Production</u>

The production of cotton seed for TwinLink™ Cotton will be conducted consistent with these standard practices; consequently, no changes to seed production practices are required. Based on the data provided by BCS for TwinLink™ Cotton (Scott, 2008), as well as previous experience with other Bt cotton varieties that have been widely adopted by growers since their introduction in 1996 (USDA-ERS, 2010a), APHIS has concluded that the availability of TwinLink™ Cotton would not alter the agronomic practices, locations, and seed production and quality characteristics of conventional and GE seed production (USDA-APHIS, 2010b). The deregulation of TwinLink™ Cotton will not require a change to seed production practices.

<u>Cumulative Impacts: Seed Production</u>

Based on current acreage trends, GE cotton varieties will likely continue to dominate. To the extent that growers see value in the traits offered by TwinLink™ Cotton, this new variety may replace existing cotton varieties, both conventional as well as GE. The availability of TwinLink™ Cotton is not anticipated to change cultivation areas for cotton production in the U.S. Because changes in the agronomic practices and locations for cotton seed production using TwinLink™ Cotton are not expected, no cumulative effects have been identified for seed production.

4.2.4 Organic Farming

Organic production plans prepared pursuant to the National Organic Program (NOP) include practical methods to protect organically-produced crops from accidental contamination with GE materials. Typically, organic growers use more than one method to prevent unwanted material from entering their fields including: isolation of the farm, physical barriers or buffer zones between organic production and non-organic production, as well as formal communications between neighboring farms (Baier, 2008; Guerena and Sullivan, 2003; NCAT, 2003). These practices follow the same system utilized for the cultivation of certified seed under the AOSCA procedures.

APHIS recognizes that producers of non-GE cotton, particularly producers who sell their products to markets sensitive to genetically engineered traits (e.g., organic or some export markets), can be reasonably assumed to be using practices on their farm to protect their crop from unwanted substances and thus maintain their price premium. APHIS will assume that growers of organic cotton are already using, or have the ability to use, these common practices as APHIS's baseline for the analysis of the alternatives.

No Action: Organic Farming

Current availability of seed for conventional (both GE and non-GE) cotton varieties, and those cotton varieties that are developed for organic production, is expected to remain the same under the No Action Alternative. Commercial production of conventional and organic cotton is not expected to change and will likely remain the same under the No Action Alternative. Planting and production of GE, non-GE, and organic cotton will continue to fluctuate with market demands, as it has over the last 10 years, and these markets are likely to continue to fluctuate under the No Action Alternative (USDA-ERS, 2010c, 2010e).

Preferred Alternative: Organic Farming

Transgenic cotton lines including those that are resistant to lepidopteran insects and herbicide-tolerant are already in widespread use by farmers. TwinLink™ Cotton should not present any new and different issues and impacts for organic and other specialty cotton producers and consumers.

Organic producers employ a variety of measures to manage identity and preserve the integrity of organic production systems (NCAT, 2003). The trend in the cultivation of GE cotton, non-GE, and organic cotton varieties, and the corresponding production systems to maintain varietal integrity, are likely to remain the same as the No Action Alternative.

According to the petition, agronomic trials conducted between 2005 and 2008 in a variety of locations in the U.S. demonstrated that TwinLink™ Cotton is not significantly different in plant growth, yield, and reproductive capacity from its nontransgenic counterpart (USDA-APHIS, 2010b). No differences were observed in pollen diameter, weight, and viability. Therefore, TwinLink™ Cotton is expected to present a similar risk of cross-pollination as existing cotton cultivars. The practices currently employed to preserve and maintain purity of organic production systems would not be required to change to accommodate the production of TwinLink™ Cotton.

Historically, organic cotton production represents a small percentage (approximately, 0.1%) of total U.S. cotton acreage (USDA-ERS, 2010e). It will likely remain small regardless of whether new varieties of GE or non-GE cotton varieties, including TwinLink™ Cotton, become available for commercial cotton production.

Accordingly, the deregulation of TwinLink™ Cotton is not expected to have a significant impact on organic cotton production.

Cumulative Effects: Organic Farming

A determination of nonregulated status of TwinLink™ Cotton is not expected to change the market demands for GE cotton or cotton produced using organic methods. A determination of nonregulated status of TwinLink™ Cotton could add another GE cotton variety to the conventional cotton market. Based upon recent trend information, adding GE varieties to the market is not related to the ability of organic production systems to maintain their market share. Although 12 GE cotton events or lines were deregulated pursuant to Part 340 and the Plant Protection Act, between 2000 and 2008, the total acreage associated with the organic production of cotton remained at slightly above 15,000 acres (USDA-ERS, 2010e).

4.2.5 Specialty Cotton Production

Specialty crop growers employ practices and standards for seed production, cultivation, and product handling and processing to ensure that their products are not pollinated by or commingled with conventional or genetically engineered crops (Bradford, 2006). These management practices include maintaining isolation distances to prevent pollen movement from other cotton sources, planting border or barrier rows to intercept pollen, and employing natural barriers to pollen (Bradford, 2006; NCAT, 2003; Wozniak, 2002). These management practices allow the grower to meet standards for the production of specialty crop seed, maintain genetic purity, and protect the genetic diversity of cotton (Bradford, 2006).

No Action: Specialty Systems

Current availability of seed for specialty cotton varieties are expected to remain the same under the No Action Alternative.

Preferred Alternative: Specialty Systems

As noted in the discussion of Seed Production, no changes in the production of seed or cultivation of cotton are required to accommodate TwinLink™ Cotton.

It is unlikely that specialty system farmers, or other farmers who choose to plant nontransgenic varieties or sell nontransgenic seed, will be substantially impacted by the expected commercial use of TwinLink™ Cotton. Transgenic cotton lines including those that are resistant to lepidopteran insects and express herbicide tolerance are already in widespread use by farmers. TwinLink™ Cotton should not present any new and different issues and impacts for specialty cotton producers and consumers.

According to the petition, agronomic trials conducted between 2005 and 2008 in a variety of locations in the U.S. demonstrated that TwinLink™ Cotton is not significantly different in plant growth, yield, and reproductive capacity from its nontransgenic counterpart (USDA-APHIS, 2010b). No differences were observed in pollen diameter, weight, and viability. Therefore, TwinLink™ Cotton is expected to present a similar risk of cross-pollination as existing cotton cultivars including other GE cotton varieties. The practices currently employed to preserve and maintain purity of specialty cotton production systems would not be required to change to accommodate the production of TwinLink™ Cotton. A determination of nonregulated status of TwinLink™ Cotton under the Preferred Alternative would not change the availability and genetic purity of seed for specialty cotton varieties. Conventional management practices and procedures, as described previously for cotton seed production, proper seed handling, protection of wild relatives of cotton, and organic cotton farming, are in place to maintain the genetic diversity of cotton. Cotton growers have utilized these methods effectively to meet the standards for the production of specialty crop seed. Impacts would be similar to the No Action Alternative.

Cumulative Effects: Specialty Systems

A determination of nonregulated status of TwinLink™ Cotton is not expected to change the market demands for GE cotton or cotton produced using specialty systems. A determination of nonregulated status of TwinLink™ Cotton will add another GE cotton variety to the cotton market. Between 2000 and 2009, 12 GE cotton events or lines were deregulated pursuant to Part 340 and the Plant Protection Act. Based on demonstrated agronomic characteristics and cultivation practices, and because the market share of specialty cotton varieties is unlikely to change by the introduction of TwinLink™ Cotton, APHIS has determined that there are no past, present, or reasonably foreseeable changes that would impact specialty cotton producers and consumers.

4.3 PHYSICAL ENVIRONMENT

4.3.1 Water Resources

Although cotton has been developed as a drought-tolerant crop (Cotton Incorporated, 2010b), irrigation (used on approximately 35% of the U.S. cotton acreage) produces much higher cotton yields (Cotton Incorporated, 2010a). Despite high production levels in irrigated areas, the lack of affordable water has been noted as one factor in the reduction of acres of cotton grown in California, Arizona, and New Mexico in the past decade (Cotton Incorporated, 2010b). In these areas, cotton has been displaced by higher value crops and land uses (Cotton Incorporated, 2010b).

Water quality is also preserved in modern cotton productions systems. The increase in conservation tillage practices has resulted in a reduction of runoff from agricultural lands, decreasing non-point source pollution of suspended sediment, fertilizer, and pesticides. Intensive monitoring of surface water and groundwater proximate to agricultural fields has demonstrated the benefits of no-till cotton in protecting both ground and surface water resources (University of Tennessee Agricultural Extension Service, 2010). Better nutrient management and precision technologies are ensuring inputs are used by the crop and are not entering ground or surface waters (Cotton Incorporated, 2010a).

No Action: Water Use

Under the No Action Alternative, current land acreage and agronomic practices, including irrigation, tillage, and nutrient management associated with cotton production would not be expected to change. Therefore, no expected changes to water use associated with cotton production is expected for this alternative.

Preferred Alternative: Water Use

In 2010, GE cotton occupied 93% of the cotton acreage (USDA-ERS, 2010c). Conventional and GE cotton production occurs on land that is dedicated to crop production and most cotton is planted in fields that have been in crop production for years (USDA-APHIS, 2010a). The introduction of GE cotton has resulted in the adoption of conservation practices (Cotton Incorporated, 2010a). These conservation practices, including reduced tillage and precision agriculture play a significant role in water conservation and maintaining water quality by minimizing soil erosion (USDA-NRCS, 2006b).

TwinLink™ Cotton would not change cultivation practices for cotton production, nor would it increase the total acres and range of U.S. cotton production areas. Therefore, a determination of nonregulated status of TwinLink™ Cotton will not change the current use of irrigation practices in commercial cotton production. Because the TwinLink™ Cotton is expected to simply replace GE and non-GE cotton varieties already in use, the consequences of the Preferred Action Alternative on water use are the same as the No Action Alternative.

Cumulative Effect: Water Use

No cumulative effects on water use have been identified for a determination of nonregulated status of TwinLink™ Cotton. A determination of nonregulated status of TwinLink™ Cotton would not change the water use and irrigation practices used in commercial cotton production.

4.3.2 Soil

This section discusses the potential consequences of the No Action and the Preferred Alternatives on soil. The USDA Natural Resources Conservation Service (NRCS) has identified significant reductions in the loss of soil from croplands in the U.S., finding that total soil loss on highly erodible croplands and non-highly erodible cropland decreased by 39.2% from 1982 to 2003 (USDA-NRCS, 2006b). Over the last 10 years, cultivation of herbicide-tolerant cotton has contributed to this reduction in soil loss through a shift towards conservation tillage and the use of cover crops (Cotton Incorporated, 2010c).

Increases in total acres dedicated to conservation tillage have been attributed to an increased use of genetically modified crops which eliminates the need for mechanical weed control (Alabama Cooperative Extension System, 1996; McClelland et al., 2000; Towery and Werblow, 2010; USDA-NRCS, 2010).

Additional soil quality benefits from adoption of GE cotton may also be realized by reducing the risks associated with environmental spills or misapplications of chemical insecticides to the soil, and reductions in the frequency with which insecticides must be applied.

No Action: Soil

Land acreage and agronomic practices associated with traditional and existing non-regulated GE cotton production would not be expected to change in response to the No Action Alternative.

Continued adoption of existing deregulated GE cotton varieties would be expected. In 2010, GE cotton occupied 93% of the cotton acreage (USDA-ERS, 2010c). Conventional and GE cotton production occurs on land that is dedicated to crop production and most cotton is planted in fields that have been in crop production for years (USDA-APHIS, 2010a).

Current agronomic practices associated with cotton production including tillage, cultivation, applications of pesticides and fertilizer, and the use of agricultural equipment would not be expected to change if TwinLink™ Cotton is still a regulated article. To the extent that currently available GE cotton varieties can result in the grower's adoption of conservation tillage, soils would be positively affected by that adoption.

Preferred Alternative: Soil

No changes to agronomic practices typically applied in the management of conventional cotton, including other commercially available GE cotton varieties, are required for TwinLink™ Cotton.

BCS' field trial and laboratory analyses demonstrated that the agronomic performance of TwinLink™ Cotton was functionally identical to its non-transgenic counterpart, the Coker 315 cultivar (Scott, 2008). No increases in fertilizers and pesticides were required, nor were any changes in cultivation, planting, harvesting, and volunteer control required (Scott, 2008). It is expected that similar agronomic practices that are currently used for commercially available GE cotton will also be used by growers of TwinLink™ Cotton. If TwinLink™ Cotton is adopted and replaces non-GE cotton varieties, soils currently under non-GE cotton production may benefit from the use of TwinLink™ Cotton due to a reduction in applications and the number of acre-treatments per year required for both insecticide and herbicide applications. Soils would also benefit from the reduction in soil disturbance because of fewer passes by heavy farm equipment.

It is expected that only glufosinate ammonium would be applied as a post-emergent herbicide on TwinLink™ Cotton. Glufosinate ammonium has been shown to rapidly dissipate from most agricultural ecosystems across a wide range of soil and climatic conditions, with a median soil half-life (the time it takes for half of the glufosinate ammonium to dissipate in the soil) of 7.5 days (PPDB, 2010). When used in accordance with the label, glufosinate ammonium accumulation in soil has not been shown to be significant.

The *bar* gene which has been introduced into TwinLink™ Cotton was originally isolated from *Streptomyces hygroscopius*, a common soil bacteria (Scott, 2008). The PAT enzyme produced by the TwinLink™ Cotton is naturally occurring in the soil (CFIA, 2004). The cultivation of TwinLink™ Cotton and the attendant production of the PAT enzyme will therefore not cause an impact to the physicochemical characteristics of the soil.

As explained above, if TwinLink™ Cotton is adopted and cultivated, there may be positive impacts to soil when compared with traditional cotton cultivation practices.

Cumulative Effects: Soil

APHIS has not identified any cumulative effects of the use of TwinLink™ Cotton to soils. Bayer has compared phenotypic, agronomic, and cultivation characteristics between TwinLink™

Cotton and control cottons (Scott, 2008). TwinLink™ Cotton required the same soil, fertilizer, water and pest management practices (except for lepidopteran pest control) as non-GE cotton (Scott, 2008). Consequently, the phenotypic, agronomic, and ecological data presented by BCS (Scott, 2008) support the conclusion by APHIS that TwinLink™ Cotton will not result in any significant impact to the soil that is not already found in conventional cotton production practices.

Based on these findings, and because the amount of cotton grown in the U.S. is unlikely to change by the introduction of TwinLink™ Cotton, APHIS has determined that there are no cumulative impacts to soil.

4.3.3 Air Quality

Traditional agricultural practices have the potential to cause negative impacts to air quality. Agricultural emission sources include smoke from agricultural burning, tillage, heavy equipment emissions, pesticide drift from spraying, and nitrous oxide emissions from the use of nitrogen fertilizer (Aneja et al., 2009; Hoeft et al., 2000; USDA-NRCS, 2006c).

The adoption of GE cotton has reduced air emissions from several of these sources. Conservation practices, including conservation tillage associated with GE cotton production requires fewer tractor passes across a field, thus decreasing dust generation and tractor emissions; surface residues and untilled organic matter physically hold the soil in place thus decreasing wind erosion of soils and pesticide drift in wind-eroded soils (Baker et al., 2005; Cotton Incorporated, 2010d; USDA-NRCS, 2006c). Reduced tillage also increases sequestration rates of carbon emissions in soils (Causarano et al., 2006). This section discusses the potential consequences of the No Action and the Preferred Alternatives on air quality.

No Action: Air Quality

Under the No Action Alternative, current impacts to air quality associated with land acreage and cultivation practices associated with cotton production would not be affected.

Adoption of GE cotton varieties would be expected to continue. To the extent that the adoption and cultivation of GE cotton varieties allows the grower to implement conservation practices, air quality improvement associated with these practices would be expected to follow. Air quality would continue to be affected by agronomic practices associated with conventional methods of cotton production such as tillage, cultivation, pesticide and fertilizer applications, and the use of agricultural equipment.

Preferred Alternative: Air Quality

TwinLink™ Cotton production does not change land acreage or any cultivation practices for conventional, transgenic, or non-transgenic cotton production. There are no expected increases in cultivation, planting, pesticide use, fertilizer use, harvesting, or volunteer control compared to currently available GE and non-GE cotton cultivars. It is expected that similar agronomic practices that are currently used for commercially available Bt cotton or herbicide-tolerant cotton will also be used by growers of TwinLink™ Cotton.

If TwinLink™ Cotton is adopted and replaces non-GE cotton varieties, air quality issues associated with pesticide application and use in non-GE cotton production may benefit from the use of TwinLink™ Cotton due to a reduction in the application of lepidopteran targeted insecticides and similar reductions in herbicide applications required as growers take advantage of the post-emergent herbicide glufosinate ammonium. The collective impact is a reduction in the number of acre-treatments per year requiring the use of heavy farm equipment, with a positive impact on air quality associated with the decrease in equipment-related emissions, as well as a decrease in dusts and pesticide drifts.

Based on this information, APHIS concludes that the production of TwinLink™ Cotton is not expected to adversely affect air quality and, in fact, may provide some benefit. However, considering the wide adoption of similar GE cotton lines, overall impacts are likely to be similar to the No Action Alternative.

Cumulative Effects: Air Quality

Based on the findings described above, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action that would have a negative impact on air quality. The consequences of the Preferred Action Alternative on commercial cotton production and the resulting air quality are similar to those expected for the No Action Alternative.

4.3.4 Climate Change

Agriculture, including land-use changes for farming, is responsible for an estimated 6% of all human-induced GHG emissions in the U.S. Moreover, nitrous oxide (N_2O) emissions from agricultural soil management (primarily fertilizer use) represent 68% of all U.S. N_2O emissions (US-EPA, 2010b). A more comprehensive discussion of the contribution of agricultural practices to GHGs is provided in Subsection 2.3.4, Affected Environment – Climate Change. Agriculture-related GHG emissions include carbon dioxide (CO_2), N_2O, and methane (CH_4), produced through the combustion of fossil fuels to run farm equipment; the use of fertilizers; or the decomposition of agricultural waste products including crop residues, animal wastes, and enteric emissions from livestock. This section discusses the potential consequences of the No Action and the Preferred Alternatives on climate change.

No Action: Climate Change

Under the No Action Alternative, environmental releases of TwinLink™ Cotton would be under APHIS regulation. Due to the limited size of these field trials, there would be no measurable effect on climate change from these confined environmental releases. Land acreage and cultivation practices associated with cotton production would not be affected. Current agronomic practices associated with conventional cotton production and current GE cotton varieties which contribute to GHG emissions, including tillage, cultivation, irrigation, pesticide application, fertilizer applications and use of agriculture equipment, would not be expected to change if TwinLink™ Cotton remains a regulated article. To the extent that the adoption and cultivation of GE cotton varieties allows the grower to implement conservation practices, GHG emissions would be expected to be reduced commensurate with the air quality improvements anticipated from adoption of conservation tillage practices.

Preferred Alternative: Climate Change

A determination of nonregulated status of TwinLink™ Cotton would not change the cultivation or agronomic practices, or agricultural land acreage associated with growing cotton, and thus is expected to have the same effect on climate change as the No Action Alternative.

Cumulative Effects: Climate Change

APHIS has not identified any cumulative effects for this issue. The use of TwinLink™ Cotton in commercial cotton production is not expected to cause any cumulative effect on climate change because APHIS does not anticipate any changes in cotton production practices or an expansion of cotton acreage as a result of TwinLink™ Cotton deregulation. The consequences of the Preferred Action Alternative on commercial cotton production and acreage are the same as for the No Action Alternative.

4.4 ANIMAL AND PLANT COMMUNITIES

4.4.1 Animals

Cotton production systems in agriculture are host to many animal species. A variety of insects feed on cotton plants or prey upon other insects inhabiting cotton fields. In addition, mammal and bird species use cotton fields and adjacent vegetation for food and habitat throughout the year. This section discusses the potential consequences of the No Action and the Preferred Alternatives on animal populations associated with cotton production. The cumulative effects analysis for this issue is found discussed below under “Cumulative Effects: Animals, Plants, and Biodiversity” in Subsection 4.4.3.

No Action: Animals

Under the No Action Alternative, conventional and GE transgenic cotton production will continue while TwinLink™ Cotton remains a regulated article. Potential impacts of GE and non-GE cotton production practices on non-target species would be unchanged.

Preferred Alternative: Animals

BCS data indicates that the agronomic practices used to produce TwinLink™ Cotton will be the same as those used to produce other conventionally grown GE and non-GE cotton. TwinLink™ Cotton production will not change land acreage or any cultivation practices for cotton production.

Since the mid-1990s, transgenic cotton lines have been commercialized without substantiated reports of significant deleterious impacts on non-target organisms (Mendelsohn et al., 2003; OECD, 2007; US-EPA, 2008a; USDA-APHIS, 2010a). Evidence submitted by BCS from laboratory and field studies as well as other peer reviewed studies demonstrate a lack of toxicity of the TwinLink™ Cotton on non-target representative species (Scott, 2008).

BCS has presented laboratory test data with indicator test species to determine potential toxicity of Cry enzyme toxin at doses higher than would be anticipated under field conditions (Rose et al., 2007). BCS' selection of representative indicator test species was based upon their potential for exposure to the Cry1Ab and Cry2Ae proteins. BCS submitted non-target data for two above-ground arthropods (Ladybug, *Coleomegilla maculate;* and Lacewing, *Chrysoperla carnea*); a soil-dwelling insect (springtail, *Folsomia candida*); a pollinator (honeybee, *Apis mellifera*); a mammal (house mouse, *Mus musculus*); and an aquatic invertebrate (water flea, *Daphnia magna*) (Scott, 2008). The data submitted in the petition indicate that no significant adverse effects were observed at the maximum test dose for either of the two Cry proteins for any of the tested species. Other research has also shown no direct adverse effects on insectivorous insects in field and laboratory studies with transgenic plants expressing Cry proteins (Marvier et al., 2007; Naranjo, 2009b; Romeis et al., 2006; Shelton et al., 2009).

The non-target risks associated with exposure of aquatic organisms is likely very low because cotton pollen is large, sticky, and is not transported long distances by the wind (USDA-APHIS, 2010a). Seed and plant debris are not expected to be readily transported via overland runoff or wind to aquatic habitats (USDA-APHIS, 2010a). Moreover, because the specific intestinal and midgut receptors for the Bt endotoxin are not present in vertebrates, these insecticidal proteins are not expected to adversely affect these organisms (Hofmann et al., 1988a; Hofmann et al., 1988b; Shimada et al., 2006b; US-EPA, 2008a; Van Rie et al., 1989; Van Rie et al., 1990).

An additional advantage of the use of transgenic cotton producing the Bt proteins is the corresponding reduction in use of broad spectrum insecticides[1] and the consequent reduction of potential impact on diversity of non-target insects (Cattaneo et al., 2006; Dively, 2005; Marvier et al., 2007; Naranjo, 2005a; Romeis et al., 2006; Torres and Ruberson, 2005; Torres et al., 2006; Whitehouse et al., 2005). Several authors note that insect-resistant crops had a higher diversity and abundance of non-target animals than conventionally grown crops (Hardee et al., 2001; Moar et al., 2003; Sanvido et al., 2006; Sisterson et al., 2007).

BCS has filed applications with the FDA and the EPA for the TwinLink™ Cotton variety. The FDA and EPA are currently reviewing the information submitted by the applicant. Both agencies have previously reviewed and approved comparable products, including several crop varieties expressing the Bt-based Cry proteins as plant-incorporated protectants, as well as the *bar* gene, resulting in the expression of the PAT enzyme providing tolerance to glufosinate

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[1] Broad spectrum insecticides are chemical insecticides which kill insects that are causing injury to plants and also kill other insects that are not causing injury to the plant. Insects that are inadvertently killed by the application of insecticide are called "non-target" insects. Because the Cry proteins are specific for a narrow range of lepidopteran insects, use of Cry proteins to control plant pests is recognized as being beneficial to the survival of non-target insects (US-EPA, 2008a).

ammonium. In the absence of a completed FDA and EPA determinations, APHIS takes into consideration prior agency reviews of comparable products to conduct its assessment of the potential impacts.

EPA has issued a tolerance exemption for Cry1Ab protein in all crops (40 CFR §174.511; US-EPA, 2010d), as well as for the PAT protein (40 CFR §174.522; US-EPA, 2010g). A temporary exemption from the requirement of a tolerance has been issued for Cry2Ae (40 CFR §174.530; US-EPA, 2010e).

The PAT enzyme *bar* gene construct was deregulated by APHIS in 2003 in the BCS LibertyLink® Cotton (USDA-APHIS, 2003). In 2003, the FDA completed its food safety consultation on LibertyLink® Cotton and granted tolerance exemptions for both the plant-incorporated protectant, as well as the genetic material necessary for its production (US-FDA, 2003). The EPA granted exemptions from the requirement of a tolerance for residues of glufosinate ammonium, as well as the plant-pesticide PAT in or on food and feed commodities of cotton (see: 62 FR 17719; 40 CFR §180.473; US-EPA, 2010c).

The conclusions presented by the EPA and the FDA were based upon their independent reviews of human health and environmental impacts associated with exposure to and consumption of plant material incorporating the *bar* gene and expressing the PAT enzyme. In both cases, these agencies determined that the cottonseed, refined oil, and cotton seed meal derived from the LibertyLink® variety was not materially different in composition, safety and nutritional value from other cotton varieties then on the market.

Similar international reviews and approvals have been published for the LibertyLink® Cotton. The Australia and New Zealand Food Authority (ANZFA) completed a Toxicological Review and Risk Assessment, and a Technical Report was published in 2005 in which ANZFA concluded that LibertyLink® Cotton was comparable to non-genetically engineered (non-GE) cotton in terms of their safety and nutritional adequacy (ANZFA, 2005). In 2004, the Plant Biosafety Office (PBO) of the Canadian Food Inspection Agency (CFIA) also concluded that BCS' LibertyLink® Cotton was substantially equivalent to currently grown cotton in terms of their potential environmental impact and livestock feed safety, and the novel traits would not have any substantial negative effect on the environment (CFIA, 2004). The CFIA specifically noted that the PAT enzyme responsible for glufosinate ammonium tolerance has very specific enzymatic activity associated with the acetylation of glufosinate ammonium, and is rapidly inactivated in mammalian stomach and intestinal fluids by enzymatic degradation and pH mediated proteolysis (CFIA, 2004).

To the extent that TwinLink™ Cotton displaces conventional, non-GE cotton varieties, the expression of the stacked Cry proteins providing lepidopteran resistance in conjunction with the tolerance to glufosinate ammonium may have an overall positive impact on animals. As previously discussed in the analysis of water, soil, in those fields where TwinLink™ Cotton is cultivated, growers would be expected to reduce their use of pesticides for control of lepidopteran pests and take advantage of the weed control offered by glufosinate ammonium, thus allowing for a shift to increased conservation practices.

The use of insecticides other than Bt crops may affect non-target organisms including honey bees, soil invertebrates, or culturable microbial flora (Dively and Rose, 2003; US-EPA, 2010a). The cultivation of insect-resistant crops allows a reduction and replacement of broad-spectrum insecticides, with a corresponding positive effect on biodiversity, including non-target insects (Cantrell, 2006). A notable advantage of GE insecticidal (Bt) crops over conventional insecticides is the high specificity of the Bt toxins, which minimize the potential toxic effects on non-target insects (Bigler et al., 2008; Sanvido et al., 2006).

The incorporation of glufosinate ammonium tolerance provides several potential advantages over conventional weed management programs, including increased flexibility to manage problem weeds, reduced use of soil-applied herbicides, reduced total use of herbicides, and more widespread adoption of conservation tillage (O'Sullivan and Sikkema, 2004). The associated adoption of conservation tillage and reduced use of soil-applied herbicides have the potential to positively impact animal populations in fields planted with TwinLink™ Cotton (see, e.g., Eggert et al., 2004).

Based on the above information, APHIS concludes that the deregulation and cultivation of TwinLink™ Cotton is expected to have no adverse effects on non-target animals. Overall impacts would be similar to the No Action Alternative.

4.4.2 Plants

The landscape surrounding a cotton field may be bordered by other cotton (or any other crop) fields or may also be surrounded by woodland, rangelands, and/or pasture/grassland areas. These plant communities represent natural or managed plant buffers for the control of soil and wind erosion and also serve as habitats for a variety of transient and non-transient wildlife species. The potential impacts to off-site plant communities is discussed in Subsection 4.4.3, Biological Diversity.

Weed control programs are important aspects of cotton cultivation. In this context, weeds are those plants which, when growing in the cotton field, compete with the cotton for space, water, nutrients, and sunlight, and may thus include native species (University of California IPM Online, 2008b). The types of weeds in and around a cotton field will vary depending on the geographic region where the cotton is grown. Common weeds in cotton include grasses, broad-leaf weeds, and sedges (*Cyperus* spp.). Some of these have been discussed in Subsection 4.2.2, Cropping Practices. This section discusses the potential consequences of the No Action and the Preferred Alternatives on plants. The cumulative effects analysis for this issue is discussed below under “Cumulative Effects: Animals, Plants, and Biodiversity” in Subsection 4.4.3.

<u>No Action: Plants</u>

Under the No Action Alternative, environmental releases of TwinLink™ Cotton would be under APHIS regulation. Plant species (i.e., weeds) that typically inhabit GE and non-GE cotton production systems will continue to be managed through the use of mechanical, cultural, and chemical control methods.

Preferred Alternative: Plants

In the event of a determination of nonregulated status of TwinLink™ Cotton , the risks to wild plants and agricultural productivity from weedy cotton populations are low; volunteer cotton populations are easily managed and feral populations occur rarely in the U.S. Cotton Belt (Wozniak, 2002).

Agronomic studies conducted by BCS tested the hypothesis that the weediness potential of TwinLink™ Cotton is unchanged with respect to conventional cotton (Scott, 2008). No differences were detected between TwinLink™ Cotton and nontransgenic cotton in growth, reproduction, or interactions with pests and diseases, other than the intended effect of protection from lepidopteran pests. The main natural controls on feral populations of cotton are poor seed dispersal, poor seed germination, competition from other plants, and a high requirement for moisture (US-EPA, 2001b). Therefore, the incorporation of either the insect resistance trait or the herbicide tolerance trait is unlikely to appreciably improve seedling establishment and increase weediness potential. Any TwinLink™ Cotton volunteers could be readily controlled by other readily available herbicides, including glyphosate (Miller et al., 2004; Stewart et al., 2003).

The incorporation of glufosinate ammonium tolerance provides several potential advantages over conventional weed management programs, including increased flexibility to manage problem weeds, reduced use of soil-applied herbicides, reduced total use of herbicides, and more widespread adoption of conservation tillage (O'Sullivan and Sikkema, 2004). The associated adoption of conservation tillage and reduced use of herbicides have the potential to positively impact plant populations in fields and fallow areas adjacent to those planted with TwinLink™ Cotton (see, e.g., Eggert et al., 2004).

The effect of cotton production on plant communities is likely to be unchanged by the introduction of TwinLink™ Cotton. Overall impacts would be similar to the No Action Alternative.

4.4.3 Biological Diversity

Biodiversity in an agroecosystem depends on four primary characteristics: 1) diversity of vegetation within and around the agroecosystem; 2) permanence of various crops within the system; 3) intensity of management, including selection and use of insecticides and herbicides; and 4) extent of isolation of the agroecosystem from natural vegetation (Altieri, 1999). The introduction of woodlots, fencerows, hedgerows, wetlands, etc. is one way to enhance biodiversity in large scale monocultures. Additional enhancement strategies include intercropping (the planting of two or more crops simultaneously to occupy the same field), agroforestry, crop rotations, cover crops, no-tillage, composting, green manuring (growing a crop specifically for the purpose of incorporating it into the soil in order to provide nutrients and organic matter), addition of organic matter (compost, green manure, animal manure, etc.), as well as the introduction of hedgerows and windbreaks (Altieri, 1999). To some degree these practices currently are being utilized by cotton growers to increase biodiversity (Cotton Incorporated, 2010a). The adoption of GE crops, with the concomitant reduction in insecticide use and enhanced soil conservation practices, has also contributed to the increase in biodiversity of soil microorganisms, beneficial organisms, and plants (Dively and Rose, 2003; Naranjo, 2009a; Sanvido et al., 2006).

No Action: Biological Diversity

Under the No Action Alternative, TwinLink™ Cotton would continue to be a regulated article. Growers and other parties who are involved in production, handling, processing, or consumption of cotton would continue to have access to existing deregulated GE lepidopteran-resistant (Bt) cotton products, herbicide-tolerant cotton products, stacked varieties presenting both, and conventional cotton varieties. The implications of agronomic practices associated with cotton production, whether traditional or GE varieties, would not change.

Preferred Alternative: Biological Diversity

The use of genetically modified cotton varieties currently available affects biological diversity by: 1) providing the opportunity to use conservation practices that enhance habitat creation; and 2) decreasing the use of pesticides which in turn, limits the impact on the floral and faunal communities that can potentially inhabit the fields and immediate surroundings (Cotton Incorporated, 2010a; Naranjo, 2009a; Sanvido et al., 2006). Incorporation of herbicide tolerance in the crop allows the grower to adopt the use of no-till, cover crops, crop rotation, intercropping, and good ditch/border/hedgerow management, each of which contributes to biodiversity in and around cotton fields (Palmer and Bromley, 2010; Sharpe, 2010). The use of broad-spectrum insecticides is one of the most severe constraints for biological diversity in crops (USDA-APHIS, 2010a). One of the benefits of Bt cotton has been the reduction of broad-spectrum insecticide use during cotton production (Fernandez-Cornejo and Caswell, 2006).

A comparison of non-transgenic and transgenic cotton production revealed no substantial impact on ant and beetle diversity in cotton fields; by contrast, broad-spectrum insecticide use demonstrated considerably reduced diversity in these populations (Cattaneo et al., 2006). Cotton expressing the Bt Cry proteins allows for the reduction in use of these broad spectrum insecticides, and is not expected to present a risk to beneficial insects and non-target pests. Research in Arizona has shown that Bt cotton has a minimum effect on both the number and function of foliar-dwelling arthropod natural enemies; on the other hand, broad-spectrum insecticide use caused a 48% reduction in 13 of the 22 taxa that were studied (Naranjo, 2005a, 2005b).

Bt toxin has been found to be present in every major part of Bt cotton plants (leaves, stems, and roots) (Vadakattu and Watson, 2004). Bt cotton roots were also found to release the Bt protein into the soil (Vadakattu and Watson, 2004). The effects of Bt on non-target soil microorganisms in Bt maize and Bt cotton cultivation found that microbial biodiversity and activity was not different from that of their non-Bt counterparts (EFSA, 2008; Hönemann et al., 2008; Icoz et al., 2008; Shen et al., 2006).

Plant biodiversity associated with cotton cultivation is also an issue of concern. Although cotton fields are cultivated as monocultures to optimize cotton yield, areas surrounding a cotton field may harbor a wide variety of plants. Broad spectrum herbicides have the potential to impact off-site plant communities. Glufosinate ammonium provides opportunities to the grower to control weeds in the field with fewer applications of herbicides than in traditional cultivation systems or those systems where glyphosate resistance has emerged (University of California IPM Online, 2008b). Accordingly, adoption of a glufosinate ammonium protocol could minimize off-site impacts. The application processes for glufosinate ammonium allow the grower to apply fewer herbicides and reduce field cultivation. Reducing the number of applications of herbicides during the cotton production cycle minimizes instances of unintentional drift of these chemicals to adjacent fields, buffer zones, ditches, and fallow areas.

Although TwinLink™ Cotton does provide the grower with a new choice for a herbicide-tolerant and insect-resistant variety, cultivation of TwinLink™ Cotton does not otherwise require a change in the rates of fertilizer application, tillage, planting, or harvesting from existing commercial cotton varieties, including other GM cotton varieties providing either insect resistance, herbicide tolerance, or stacked with both (Scott, 2008). Therefore, the effect of cotton production on plant communities is likely to be unchanged by the introduction of TwinLink™ Cotton.

A determination of nonregulated status of TwinLink™ Cotton will not change the cultivation or agronomic practices, or agricultural land acreage associated with growing cotton. TwinLink™ Cotton would be an additional Bt cotton variety providing a different option for herbicide use (glufosinate ammonium) for growers to use and therefore is expected to have the same effect on biological diversity as the No Action Alternative.

Therefore, the deregulation of TwinLink™ Cotton for the control of lepidopteran pests and tolerance of glufosinate ammonium is highly unlikely to have any direct toxic effects on non-target organisms and is likely to be neutral or beneficial to animal and plant biodiversity compared to non-transgenic cotton managed with conventional broad-spectrum insecticides.

Cumulative Effects: Animals, Plants, and Biodiversity

APHIS has determined that there are no impacts from past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to create cumulative impacts or reduce the long-term productivity or sustainability of any of the resources associated with the ecosystem in which TwinLink™ Cotton is planted. Based on scientific evidence, the diversity and abundance of non-target organisms in lepidopteran-resistant cotton is at least as high as in conventional cotton; and in many studies, higher biodiversity is associated with transgenic cotton (Cattaneo et al., 2006; Head et al., 2005; Naranjo, 2005a, 2005b; Torres and Ruberson, 2007; Torres et al., 2006; Whitehouse et al., 2005).

Cultivation of TwinLink™ Cotton, with the attendant expression of the two stacked Cry proteins and the PAT enzyme providing herbicide tolerance, is highly unlikely to have direct toxic effects on non-target organisms and is likely to be neutral or beneficial to biodiversity compared with conventionally managed cotton. Therefore, the likelihood of adverse cumulative effects on non-target organisms and biodiversity following the introduction of TwinLink™ Cotton is minimal.

4.4.4 Gene Movement

Gene flow is the movement of genes from one organism to another. Vertical gene flow, or introgression, is the movement of genes to sexually compatible relatives (Ellstrand, 2003; Quist, 2010). In assessing the risk of gene introgression from TwinLink™ Cotton to its sexually compatible relatives, APHIS considered two primary issues: 1) the potential for gene flow and introgression to feral upland cotton and cotton relatives and 2) the potential impact of introgression. This is discussed below under the "Preferred Alternative" heading.

Horizontal gene flow is the stable movement of genes from one organism to another without reproduction or human intervention (Keese, 2008; Quist, 2010). There is no evidence of naturally occurring transgene movement from transgenic crops to sexually incompatible species (Scott, 2008; Stewart, 2008). Horizontal gene transfer and consequent expression of DNA from one plant to another plant or other phyla (e.g., species of bacteria) is unlikely to occur (Keese, 2008). This event would require physical relocation of the complete genetic material from the transgenic plant to the new location, including not only the genes which code for the production of specific proteins, but also those portions of the genome which regulate the activity of those genes (Keese, 2008; Scott, 2008). There are no known naturally occurring vectors (such as plasmids, phages or transposable elements) that could be responsible for inter-domain gene transfer, and there is little evidence that eukaryotic cells are naturally capable of stably incorporating genes from the environment into their genome (Brown, 2003). Although viruses do move genetic material, all viruses that infect higher plants have small RNA or DNA genomes, usually with fewer than 20 encoded proteins (Keese, 2008). These viruses are therefore constrained as to the type and size of novel genetic material which can be acquired by horizontal gene transfer (Stewart, 2008).

Two bacteria species commonly associated with plants, *Agrobacterium* and *Rhizobium,* have been evaluated to determine the probability of horizontal gene transfer between the bacterium and their host plants. *Agrobacterium* moves its genes from its bacterial plasmid to the plant causing the plant to produce the gall (abnormal outgrowth) (University of Illinois, 2010). *Rhizobium* aids in nitrogen fixation in legume nodules (Wilkinson and Elevitch, 2011). The genomes of both bacteria have been sequenced, and the sequenced genes evaluated for exogenous genes (Kaneko et al., 2000; Kaneko et al., 2002; Wood et al., 2001). Despite what would appear to be millennia of symbiotic relationships between these bacteria and their host plants, there is no evidence that these organisms contain genes derived from plants (USDA-APHIS, 2010a). In cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale in the order of millions of years (Brown, 2003; Koonin et al., 2001). Transgene DNA promoters and coding sequences are optimized for plant expression, not bacterial expression (USDA-APHIS, 2010a). Horizontal gene flow, resulting in the relocation of entire transgenes including the regulatory portions of the DNA (those parts of the DNA which code for the production of the specific proteins in that relocated transgene) has never been shown to occur in nature (Clarke, 2007; Stewart, 2008). Thus, even if horizontal gene transfer occurred, proteins associated with these transgenes are not likely to be produced in the new host organism (Stewart, 2008; USDA-APHIS, 2010a). Based on this information, APHIS considers the horizontal gene flow from TwinLink™ Cotton to unrelated species to be highly unlikely, and the same as potential horizontal gene flow from existing GE cotton varieties.

No Action: Gene Movement

Under the No Action Alternative, conventional and GE transgenic cotton production will continue while TwinLink™ Cotton will remain a regulated article. Cotton land acreage and cultivation practices for both conventional and GE transgenic cotton varieties will remain the same. In 2010, Bt cotton varieties occupied 93% of the cotton acreage (USDA-ERS, 2010c). Gene flow from current commercially available GE cultivars to non-GE cotton cultivars, feral cotton populations, and wild sexually compatible relatives will remain unchanged from the current condition.

Preferred Alternative: Gene Movement

Under this alternative, TwinLink™ Cotton would be available to growers, raising the potential for gene introgression of TwinLink™ Cotton with other sexually compatible, related species.

The passage of the transgene to a cross-compatible wild relative could allow the wild relative to become more abundant in the habitat or to invade new habitats because of its lepidopteran resistance (Wilkinson and Ford, 2007). This increased resistance of the wild relative to one group of insects could alter the balance of other insect herbivores, predators, and parasites (Wilkinson and Ford, 2007). The introgressed wild crop relative could also impact other plants through interspecific (between species) competition and indirectly affect the insect pollinators, herbivores, predators, and parasites of those species (Wilkinson and Ford, 2007). Vertical gene flow, or introgression, is influenced by many factors, including the subtle differences between flowers and pollinators in related species, the mode of seed dispersal, and habitat where the two species grow (Messeguer, 2003; Quist, 2010). Introgression can be managed by understanding these biological and physical barriers to pollen movement (Daniell, 2002; Eastham and Sweet, 2002; Messeguer, 2003; Quist, 2010).

Two species of cotton are cultivated in the U.S.: upland cotton (*G. hirsutum*) and Pima cotton (*G. barbadense*) (USDA-ERS, 2009). There are two wild relatives of interest to U.S. growers, *G. thurberi* and *G. tomentosum,* found in the mountains of southern Arizona and in Hawaii, respectively. APHIS has considered the potential for gene flow from TwinLink™ Cotton, a GE variety of *G. hirsutum*, to other upland cotton varieties, as well as these other three species.

With regard to gene flow to other upland cotton varieties (*G. hirsutum*), such gene flow is controlled by careful adherence to isolation distances, border and barrier rows, and similar practices developed following AOSCA standards for identity protection of seeds. These measures have been developed based on research on the pollination of the upland cotton flower.

Although upland cotton is considered self-pollinating (Hartman and Kester, 1975; Scott, 2008), cotton can be pollinated by bees. McGregor (1976) illustrated a rapid decline in the transfer of cotton pollen by bees over distance and found that at 150 to 200 feet away from the source plant, only 1.6% of the bees showed the presence of the source plant pollen particles. Very little pollen is transferred beyond 12 meters (McGregor, 1976; Scott, 2008; Vaissiere and Vinson, 1994). By comparison, the isolation distances for Foundation, Registered, and Certified seeds specified in 7 CFR Part 201 are 1320, 1320, and 660 feet, respectively. If these identity practices are adhered to by growers, it is unlikely that TwinLink™ Cotton will outcross to other upland cotton varieties.

Upland cotton and Pima cotton are frequently cultivated in the same regions (Hutmacher et al., 2006). Although cross pollination has been demonstrated between these species, Van Deynze and others have found that the isolation distances currently recommended to ensure seed quality for Foundation and Registered seed production are adequate to maintain varietal purity (Hutmacher et al., 2006; Van Deynze et al., 2005).

Outcrossing of genes from TwinLink™ Cotton to *G. thurberi* is unlikely to occur because *G. thurberi* contains only the D genome whereas *G. hirsutum* contains both the A and D genome. The difference in chromosome numbers precludes sexual compatibility of *G. hirsutum* with *G. thurberi* (OECD, 2008).

G. tomentosum is native to the Hawaiian Islands (Pleasants and Wendel, 2010). Historic literature suggests that differences in flowering patterns and pollinators presented barriers to cross pollination between *G. tomentosum* and either *G. hirsutum* or *G. barbadense* (Pleasants and Wendel, 2010). Recent field and laboratory studies contradict this historic literature, demonstrating that these three species share common pollinators, and further that differences in flower structure and flowering habits do not serve as barriers to cross pollination (Pleasants and Wendel, 2010). Although laboratory and greenhouse breeding programs with hand pollination have produced viable progeny, DNA marker analyses have not found evidence of natural hybridization genes between *G. hirsutum* and *G. tomentosum* (DeJoode and Wendel, 1992).

The EPA has established cultivation restrictions on Bt cotton varieties to reduce the possibility of gene transfer to feral cotton relatives in Hawaii, Florida, Puerto Rico, and the U.S. Virgin Islands (US-EPA, 2008a). The terms and conditions of EPA's conditional registration for Cry1Ab in cotton prohibits commercial cultivation south of Route 60 near Tampa (US-EPA, 2008a). TwinLink™ Cotton is neither expected to be planted commercially in the areas of Florida where wild populations of *G. hirsutum* occur nor would they likely be impacted by TwinLink™ Cotton planted north of Route 60. In Hawaii, the EPA (2001b) has restricted the sale and distribution of Bt cotton for commercial planting where it could outcross with the wild cotton species *G. tomentosum.* These restrictions in Hawaii also apply to Bt cotton test plots and breeding nurseries (US-EPA, 2001b). Because of these restrictions, gene flow from Bt cotton to wild or feral populations of *G. hirsutum* or to *G. tomentosum* is extremely unlikely.

APHIS evaluated the potential for gene introgression to occur from TwinLink™ Cotton to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Based on the plant pest risk assessment, APHIS has concluded that TwinLink™ Cotton is not a plant pest and that gene flow between this product and wild or feral relatives will not occur in the U.S. (USDA-APHIS, 2010b).

Based on the above information, APHIS has concluded that that a determination of nonregulated status of TwinLink™ Cotton will not impact sexually compatible relatives, nor would it increase the weedy or invasive characteristics of weedy or wild relatives if gene flow or introgression were to occur (USDA-APHIS, 2010b). TwinLink™ Cotton would provide growers with an additional cotton variety combining a Bt-based lepidopteran protection, as well as herbicide tolerance, and is expected to have the same effect on gene movement as the No Action Alternative.

Cumulative Effect: Gene Movement

Based on available scientific evidence, APHIS has not identified any cumulative effects on gene movement that would occur from a determination of nonregulated status of TwinLink™ Cotton.

Gene movement between sexually compatible cotton species is no greater for TwinLink™ Cotton than it is for other non-GE or GE cultivars. Many factors limit the likelihood of gene movement between cotton species. These include:

- Cotton is considered to be a self-pollinating crop with a very low frequency of outcrossing from insect pollinators or wind;
- The *Cry1Ab*, *Cry2Ae*, and *bar* genes in TwinLink™ Cotton and other GE cottons impart no agronomic advantages whereby a greater potential for weediness or invasiveness would result should introgression occur;
- Neither GE or non-GE cotton cultivars form self-sustaining populations outside of cultivation because of limitations in seed dispersal, germination, and high moisture requirements (US-EPA, 2001b, 2008a);
- The cotton industry has measures in place to restrict pollen movement and gene flow between cotton fields through the use of isolation distances, border and barrier rows, the staggering of planting dates and various seed handling, transportation and ginning procedures (Sundstrom et al., 2002); and
- Where a potential for GE cotton to introgress into wild or feral populations of cotton exists, the EPA has placed restrictions on GE cotton breeding, testing, and production (US-EPA, 2001b, 2008a).

In addition, there is no evidence that horizontal gene transfer and expression of DNA occurs between cotton and soil bacteria or unrelated plant species under natural field conditions, and even if this were to occur, proteins corresponding to the transgenes are not likely to be produced.

4.5 PUBLIC HEATH

4.5.1 Human Health

Under FFDCA, it is the responsibility of food and feed manufacturers to ensure that the products they market are safe and properly labeled. Food and feed derived from TwinLink™ Cotton must be in compliance with all applicable legal and regulatory requirements. GE organisms for food and feed may undergo a voluntary consultation process with the FDA prior to release onto the market. The EPA also has authority over GE crops such as TwinLink™ Cotton which present pesticidal properties such as Bt toxin.

With regard to human health effects, the FDA and the EPA assess the relative toxicity of the incorporated proteins on humans from the perspective of both consumption, as well as exposure. BCS has submitted a food and feed safety and nutritional assessment to the FDA, as well as a registration review to the EPA for this product. The FDA and EPA are currently reviewing the information submitted by the applicant.

No Action: Human Health

Under the No Action Alternative, TwinLink™ Cotton would continue as a regulated article. Grower and consumer exposure to this product would be limited to those individuals involved in the cultivation under regulated conditions. Human exposure to existing traditional and GE cotton would not change under this alternative.

Preferred Alternative: Human Health

APHIS considers the FDA and the EPA regulatory assessment in making its determination of the potential impacts of deregulation of the new agricultural product. BCS has submitted a food and feed safety and nutritional assessment to the FDA, as well as a registration review to the EPA for this product. The FDA and EPA are currently reviewing the information submitted by the applicant. In the absence of these completed agency reviews, APHIS takes into consideration prior reviews of comparable products as a starting point for its human health impact determination.

Similar products have been deregulated and approved for general cultivation beginning in 1996 with the introduction of Bt products and followed shortly after by the introduction of the various "Liberty" products which provided tolerance to glufosinate ammonium. In each case, FDA and EPA reviews and approvals determined that the products met the agency's review criteria for approval. The cultivation of these existing crop products would not change under either alternative. Both characteristics have been successfully cultivated in multiple crops in the ensuing years with no evidence of human health impacts.

Note that with regard to the expression of glufosinate ammonium tolerance, the gene construct in the Bayer TwinLink™ Cotton is the same as that approved by the FDA in June, 2003 for the LibertyLink® Cotton product (US-FDA, 2003). In that approval, the FDA noted that the transformational event in LibertyLink® Cotton was not materially different in composition, safety, or any other relevant parameter in cotton then grown, marketed, and consumed (US-FDA, 2003).

The EPA, in 2008, published a Biopesticides Registration Action Document (BRAD) for *Bacillus thuringiensis* (*Bt*) incorporated protectants (US-EPA, 2008a). Based on a comprehensive human health effects assessment, the Bt BRAD found no unreasonable adverse effects associated from exposure to the Bt products. The Bt protein is a registered microbial pesticide which is approved for use on food crops. The EPA concluded in the BRAD that the Bt products incorporated as plant protectants would be expected to behave as expected of a dietary protein (US-EPA, 2008a). A tolerance exemption is in place for Cry1Ab protein in all crops (40 CFR §174.511; US-EPA, 2010d), as well as for the PAT protein (40 CFR §174.522; US-EPA, 2010g). A temporary exemption from the requirement of a tolerance has been issued for Cry2Ae (40 CFR §174.530; US-EPA, 2010e).

For the majority of Bt proteins currently registered, the source bacterium has been a registered microbial pesticide, which has been approved for use on food crops without specific restrictions.

Because of their use as microbial pesticides, a long history of safe use is associated with many Bt products (US-EPA, 2001b). Results from numerous animal and human epidemiological studies together, over the years, indicate that biopesticides pose minimal risks to humans and animals (Betz et al., 2000; US-EPA, 2001b, 2008a).

The Cry proteins present in the TwinLink™ Cotton plant have been reviewed and registered by the EPA since 1996 and re-registered in 2001 and 2006 (US-EPA, 2001b, 2008a). The EPA has determined that the use of these Cry proteins as plant-incorporated protectants do not pose a risk to human health (Betz et al., 2000; US-EPA, 2001b, 2008a).

The PAT protein in TwinLink™ Cotton is derived from *Streptomyces hygroscopius*, a common soil bacteria. Humans are frequently exposed to this bacteria when consuming vegetables or root crops, and there is no evidence of harm from such exposure (Delaney et al., 2008; Hérouet et al., 2005). APHIS deregulated the PAT enzyme *bar* gene construct in cotton in 2003 with the BCS LibertyLink® Cotton (USDA-APHIS, 2003). In 2003, the FDA completed its food safety consultation on LibertyLink® Cotton and granted tolerance exemptions for both the plant-incorporated protectant, as well as the genetic material necessary for its production (US-FDA, 2003). The EPA granted exemptions from the requirement of a tolerance for residues of glufosinate ammonium, as well as the plant-pesticide PAT in or on food and feed commodities of cotton (see: 62 FR 17719; 40 CFR §180.473; US-EPA, 2010c).

BCS conducted safety evaluations based on Codex Alimentarius Commission procedures to assess any potential adverse effects to humans or animals resulting from environmental releases of TwinLink™ Cotton containing the Cry1Ab, Cry2Ae, and PAT proteins see (FAO, 2001; Scott, 2008). These safety studies included evaluating protein structure and function, including homology searches of the amino acid sequences with comparison to all known allergens and toxins, an *in vitro* digestibility assay of the proteins, and an acute toxicity test in the mouse. The TwinLink™ Cotton proteins were determined to have no amino acid sequence similar to known allergens and lacked toxic potential to mammals (Scott, 2008). The results of studies conducted by BCS confirm that the crops containing these proteins can be safely used as food or feed (Scott, 2008).

The Australia and New Zealand Food Authority (ANZFA) also completed a Toxicological Review and Risk Assessment, and a Technical Report was published in 2005 (ANZFA, 2005). ANZFA concluded that LibertyLink® Cotton was comparable to non-genetically engineered (non-GE) cotton in terms of their safety and nutritional adequacy. In 2004, the Plant Biosafety Office (PBO) of the Canadian Food Inspection Agency (CFIA) concluded that BCS's LibertyLink® Cotton was substantially equivalent to currently grown cotton, in terms of their potential environmental impact and livestock feed safety, and the novel traits would not have any substantial negative effect on the environment (CFIA, 2004). CFIA further noted that the PAT enzyme responsible for glufosinate ammonium tolerance has very specific enzymatic activity associated with the acetylation of glufosinate ammonium, and is rapidly inactivated in mammalian stomach and intestinal fluids by enzymatic degradation and pH mediated proteolysis (CFIA, 2004).

As with other Bt cotton and herbicide-tolerant cotton products previously deregulated and commercialized, including the LibertyLink® variety in 2003, TwinLink™ Cotton is expected to be used throughout cotton producing areas of the country. Based on the analysis of field and laboratory data and scientific literature provided by BCS (Scott, 2008), and safety data available on other GE cotton, APHIS has concluded that a determination of nonregulated status of TwinLink™ Cotton would have no adverse impacts on human health. Overall impacts are similar to the No Action Alternative.

Cumulative Effects: Human Health

There are no significant impacts on human or animal health related to the No Action Alternative or a determination of nonregulated status of TwinLink™ Cotton, and no cumulative effects have been identified.

4.5.2 Worker Safety

EPA's Worker Protection Standard (WPS) (40 CFR Part 170) was published in 1992 to require actions to reduce the risk of pesticide poisonings and injuries among agricultural workers and pesticide handlers. The WPS offers protections to more than two and a half million agricultural workers who work with pesticides at more than 560,000 workplaces on farms, forests, nurseries, and greenhouses. The WPS contains requirements for pesticide safety training, notification of pesticide applications, use of personal protective equipment, restricted entry intervals following pesticide application, decontamination supplies, and emergency medical assistance.

No Action: Worker Safety

During agricultural production of cotton, agricultural workers and pesticide applicators may be exposed to a variety of EPA registered pesticides (US-EPA, 2001b). Under the No Action Alternative, agricultural workers and pesticide applicators may be exposed to these agricultural chemicals during cotton production. Such chemicals would be expected to include those products currently used for insect pest and plant pest management in both GE and non-GE cotton cultivation.

Preferred Alternative: Worker Safety

Before the introduction of Bt cotton varieties in 1996, chemical pesticides were widely used to control lepidopteran pests of cotton. Organophosphate, carbamate, and pyrethroid products accounted for a substantial percentage of the insecticides used (USDA-NASS, 2008). However, these three classes of pesticides require numerous safety warnings and extensive use restrictions, which raise concerns for worker safety (US-EPA, 2001b). Bt cotton varieties have offered a very effective and environmentally benign alternative to chemical insecticides for lepidopteran insect pest control. As a result, Bt cotton varieties have been extensively adopted by cotton farmers and now represent 73% of the commercial cotton planted (USDA-ERS, 2010a). Herbicide-tolerant cotton varieties have seen similar adoption within the industry, with a 78% adoption rate in 2010 (USDA-ERS, 2010a). The adoption of herbicide tolerance has allowed growers to shift their herbicide use to products like glufosinate ammonium which has a low toxicity to humans. Avoidance of other herbicides provides corresponding benefits.

Similar to the No Action Alternative, it is expected that EPA registered pesticides that are currently used for cotton production will continue to be used by growers. If TwinLink™ Cotton is adopted, agricultural workers and pesticide applicators may benefit from the use of TwinLink™ Cotton due to a reduction in the use of budworm/bollworm insecticide applications and the use of the low-toxicity herbicide glufosinate ammonium. The adoption of glufosinate ammonium-tolerant cotton allows growers to change cultivation practices in those fields where glyphosate resistance has emerged, with the resulting potential reduction in herbicide treatments required in those current glyphosate-based systems. A reduction in insecticide and herbicide use would benefit worker safety.

Cumulative Effects: Worker Safety

A determination of nonregulated status of TwinLink™ Cotton is not expected to increase the total acreage of cotton production or the use of Bt cotton, herbicide-tolerant cotton, or stacked varieties presenting both traits. BCS anticipates that TwinLink™ Cotton will primarily replace some of the insect-resistant and herbicide-tolerant cotton cultivars already on the market today. As a result, worker safety issues related to the use of EPA registered pesticides during conventional and GM cotton production should remain the same. However, if a grower replaces a non-Bt cotton or non-herbicide-tolerant cotton variety with TwinLink™ Cotton, then it would be expected that there would be a decrease in the use of lepidopteran pest insecticides. Similarly, replacement of herbicide-tolerant cotton currently based on expressing glyphosate tolerance with TwinLink™ Cotton has the potential to reduce herbicide use to manage weedy species which are glyphosate-resistant. This has been the case with the adoption of other GM cotton cultivars (Benbrook, 2009). A reduction in pesticide use and exposure should positively benefit worker safety.

4.6 ANIMAL FEED

Cotton is grown primarily for its lint, but the seed residue, called cottonseed meal, provides a valuable animal feed (Meyer et al., 2007). Cotton seed meal animal feed provides protein, fat, and energy (National Cottonseed Products Association, 2002). Under FFDCA, it is the responsibility of feed manufacturers to ensure that the products they market are safe and properly labeled. Feed derived from TwinLink™ Cotton must be in compliance with all applicable legal and regulatory requirements. GE organisms for feed may undergo a voluntary consultation process with the FDA prior to release onto the market. BCS has submitted a food and feed safety and nutritional assessment to the FDA, as well as a registration review to the EPA for this product. The FDA and EPA are currently reviewing the information submitted by the applicant. In the absence of these completed agency reviews, APHIS takes into consideration prior reviews of comparable products as a starting point for its animal health impact determination.

No Action: Animal Feed

Under the No Action Alternative, TwinLink™ Cotton will remain a regulated product and will not be available as an animal feed.

Cotton-based animal feed will still be available from currently cultivated cotton crops, both conventional varieties as well as deregulated GE cotton expressing either insect resistance or herbicide tolerance. No change in the availability of the residues of these crops as animal feed is expected under this alternative.

Preferred Alternative: Animal Feed

BCS has submitted compositional and nutritional characteristics of TwinLink™ Cotton seed to APHIS (Scott, 2008). The nutritional components of TwinLink™ Cotton were compared with the non-transgenic cotton cultivar, Coker 315. The compositional comparison was based on analysis of products collected from seven field trials (Scott, 2008). Chemical analysis of the cotton samples included the nutritional factors proximate (moisture, crude protein, lipids, ash, carbohydrates, and energy); minerals, vitamins, amino acids, and fatty acids (cottonseed oil); and the antinutrient factors of cotton including the gossypols, phytic acid, and the cyclic fatty acids (Scott, 2008). The field trials were conducted to simulate modern cotton cultivation techniques, so that the replicates evaluated included fields treated with glufosinate ammonium in accordance with the label provisions and untreated fields (Scott, 2008).

The results provided by BCS show no differences between TwinLink™ Cotton and the non-transgenic Coker 315 cultivars; reported results for each parameter were also within the reference ranges for cotton composition (Scott, 2008). All the analytes measured fell within the normal range of natural variation in cotton (ILSI, 2008; OECD, 2004).

The EPA has established a permanent exemption from the requirement of a tolerance for the PAT enzyme and the genetic material necessary for its production in plants (40 CFR 174.522; US-EPA, 2010g). A similar permanent exemption has been published for the Cry1Ab protein in all plants (40 CFR 174.511; US-EPA 2010d). A temporary exemption for the requirement of a tolerance for the Cry2Ae protein in cotton has been published at 40 CFR 174.530, in accordance with Experimental Use Permit 264-EU-143 (US-EPA, 2010e, 2010f).

APHIS's assessment of the safety of this product for animals focuses on plant pest risk (USDA-APHIS, 2010b) and effects on wildlife and threatened and endangered species and those analyses are based on the comparison of the GE TwinLink™ Cotton to its non-GE counterpart. No new issues appear to be associated with the Cry and the PAT proteins in TwinLink™ Cotton.

Based on this information APHIS has concluded that a determination of nonregulated status of TwinLink™ Cotton would have no significant impacts on animal feed or animal health. Overall impacts are similar to the No Action Alternative.

Cumulative Effects: Animal Feed

There are no significant impacts on animal health related to the No Action Alternative or a determination of nonregulated status of TwinLink™ Cotton, and no cumulative effects have been identified.

4.7 SOCIOECONOMIC ISSUES

4.7.1 Domestic Economic Environment at Risk

Cotton producers in the U.S. are among the most technically advanced in the world, annually harvesting about 17 million bales or 7.2 billion pounds of cotton (US-EPA, 2009). The USDA ERS notes that U.S. cotton productivity has increased in recent years as a result of technological advancements, including biotechnology-derived varieties (Meyer et al., 2007). Herbicide-tolerant cotton is one of the most widely and rapidly adopted crops in the U.S., followed by insect-resistant cotton (Fernandez-Cornejo and Caswell, 2006; USDA-ERS, 2010a). By the adoption of new technologies, including agronomic traits delivered through biotechnology, the yields of cotton lint per acre in the U.S. ranks among the highest in the world (USDA-APHIS, 2010a). Bt varieties of cotton offer excellent protection from the damage incurred by insect pests and an economical alternative to broad-spectrum insecticides. In addition, the adoption of post-emergent herbicide-tolerant varieties provide opportunities to apply fewer herbicides and to reduce field cultivation (University of California IPM Online, 2009c; USDA-APHIS, 2010a).

No Action: Domestic Economic Environment

Under the No Action Alternative, TwinLink™ Cotton would continue to be regulated articles under the regulations at 7 CFR Part 340. Growers and other parties who are involved in production, handling, processing, or consumption of cotton would not have access to TwinLink™ Cotton, but would continue to have access to existing deregulated GE lepidopteran-resistant or herbicide-tolerant (or stacked) cotton varieties as well as other deregulated GE and conventional cotton varieties. Domestic growers will continue to utilize currently available traditional and GE cotton varieties based upon availability and market demand. Consequently, the economic profile of cotton production and marketing is not expected to change.

Preferred Alternative: Domestic Economic Environment

BCS anticipates that TwinLink™ Cotton would replace currently available Bt cotton varieties and may be selected by growers of conventional cotton varieties making a change to a GE variety; however, availability of TwinLink™ Cotton is not expected to result in an increase in cotton acreage (Scott, 2008). BCS considers the major benefits resulting from the introduction of TwinLink™ Cotton and its progeny to be additional grower choice, increased competition, and extended useful life of Bt cotton technology.

In its review and analysis of the public interest documents submitted to support current Bt cotton products, EPA determined that economic value would result from the sale and use of these products (US-EPA, 2001b). Other studies have reached the same conclusions. For example, based on an analysis of biotechnology-derived crops planted in 2004, the National Center for Food and Agricultural Policy estimated that products providing protection against cotton bollworm, tobacco budworm, and pink bollworm increased cotton production by almost 600 million pounds, improved farm income by almost $300 million, and reduced chemical pesticide use by more than 1.6 million pounds (Sankula et al., 2005). In 2006, Fernandez-Cornejo and Caswell provided a summary of other studies that characterize effects on yield, pesticide use, and grower returns, suggesting that in addition to the economic benefits to the GE developers and seed firms associated with technology fees and seed premiums, both U.S. and foreign consumers indirectly benefit through lower commodity prices resulting from increased supplies (Fernandez-Cornejo and Caswell, 2006). Market benefits were estimated at $230 million for herbicide-tolerant cotton and $210 million for insect-resistant cotton (Bt cotton) after only two years of availability (Fernandez-Cornejo and Caswell, 2006).

BCS' field trials demonstrated that TwinLink™ Cotton was effective in controlling lepidopteran larvae, such as cotton bollworm (CBW, *Helicoverpa zea*), tobacco budworm (TBW, *Heliothis virenscens*) larvae, and fall armyworm (FAW, *Spodoptera frugiperda*) which are common pests of cotton (Scott, 2008). These pests cause severe economic damage to the cotton crop if not controlled. If controlled by chemical pesticides, there is the need for large insecticide input annually to control these pests (Scott, 2008). The incorporation of the two Bt-based Cry proteins obviates the need for the input of these broad spectrum insecticides, with a commensurate economic benefit (reduced cost) to the grower.

Despite the wide adoption of Bt cotton varieties to control lepidopteran insects, these same species continue to be the most economically important pests of the crop. In 2009, it was estimated that the Heliothine complex of *H. virescens* and *H. zea* infested 4.4 million acres of cotton and reduced yields across the Cotton Belt by 91,119 bales (Williams, 2010). Total 2009 cost and loss estimates for arthropod cotton pests were $502 million (Williams, 2010).

BCS anticipates that TwinLink™ Cotton will replace or displace existing GE cotton varieties currently in the market. Specific economic projections are not provided. To the extent that the planting of TwinLink™ Cotton results in a decrease in insecticide applications for lepidopteran pest control and a potential decrease in herbicide applications where the utilization of glufosinate ammonium allows growers to reduce or eliminate multiple herbicides to control glyphosate-resistant weeds, farms adopting TwinLink™ Cotton might experience an increase in net income. However, net income differentials cannot be projected. The net income of cotton producers just growing Bt cotton varieties or herbicide-tolerant varieties is not available (USDA-NASS, 2007). However, as BCS anticipates that TwinLink™ Cotton would only replace existing GE cotton varieties and not result in increased cotton acreage, resulting socioeconomic impacts are expected to be similar to the No Action Alternative.

Cumulative Effects: Domestic Economic Environment

Based on the information described above, APHIS concludes that a determination of non-regulated status of TwinLink™ Cotton in itself will have no foreseeable adverse cumulative domestic economic effects. TwinLink™ Cotton would simply replace or displace existing cotton varieties for those growers seeking a new combination of insect resistance and herbicide tolerance. TwinLink™ Cotton is not expected to lead to an expansion of U.S. cotton acreage. No net cumulative effects have been identified associated with a determination of non-regulated status and cultivation of TwinLink™ Cotton.

4.7.2 Trade Economic Environment at Risk

Cotton is the single most important textile fiber in the world, accounting for about 40% of all fibers produced. On average, the U.S. produces 20% of the global cotton production and is the leading supplier in the international market (Meyer et al., 2007). However, the U.S. cotton sector has faced a number of challenges as it shifts from a domestic-oriented market to one focused largely on the global marketplace. Domestic mill demand has declined significantly (in the U.S.) from only a decade ago as competition from imported textile and apparel products has risen dramatically. Meanwhile, export demand has increased rapidly with the recent expansion of global textile production (Meyer et al., 2007). The USDA ERS reports that U.S. cotton mills consumed 60% of the domestic cotton through the 1990s, but by the mid-2000s 70% of the U.S. cotton was exported (Meyer et al., 2007). As a result, U.S. cotton prices are no longer determined solely by domestic supplies and stocks (Isengildina-Massa and MacDonald, 2009; Meyer et al., 2007).

Research using a multi-region computable general equilibrium model assessed the impacts of international Bt cotton adoption on cotton and related sectors of regional economies (Frisvold and Reeves, 2007). Productivity gain estimates were based on 2005 adoption rates for Bt cotton in seven countries. Global economic benefits were nearly $1.4 billion, with U.S. benefits being over $200 million. Increased production from Bt cotton adoption led to a 3% reduction in the world cotton price. Employment and trade balances in the textile and apparel sectors increased for China and India, but generally declined elsewhere. Individual countries obtained greater economic welfare gains upon adoption of Bt cotton; non-adopting regions lost cotton market share to adopting regions (Frisvold and Reeves, 2007).

U.S. cotton production reached consecutive records in 2004 and 2005 seasons, with rising global cotton demand providing a market for much of the increased output (Meyer et al., 2007). However, the growing use of better crop production technologies overseas may narrow the gap between foreign production and mill use, constraining growth in foreign import demand and U.S. cotton exports (Meyer et al., 2007). Beginning in the 2006 growing year, U.S. cotton acreage was considerably lower, as prices favored the planting of alternative crops such as corn and soybeans (USDA-ERS, 2009). Increased acreage in cotton in 2010 was attributed to an export ban imposed by India early that same year (USDA-FAS, 2010a). Meanwhile, debate over trade policy and the sustainability of current farm programs are a source of uncertainty for U.S. agricultural commodities in general and the cotton sector in particular (Meyer et al., 2007).

Around the world, new technology has made cotton more attractive to farmers in many countries, and policy reforms in other countries have increased farmers' willingness to plant cotton (USDA-ERS, 2009). Outside the U.S., the spread of Bt cotton has recently revolutionized India's cotton sector as had occurred in China (Meyer et al., 2007). The cost savings of Bt cotton brought millions of hectares back into cotton production in eastern China and has also helped India's cotton area rebound by more than 1 million hectares (Meyer et al., 2007). Bt cotton has also been adopted in smaller producing countries like Australia, Argentina, Mexico, and South Africa (Meyer et al., 2007; USDA-ERS, 2009).

No Action: Trade Economic Environment

The cropping and marketing decisions made by cotton growers are unlikely to be influenced by the selection of this alternative and it is expected that approximately 93% of the cotton produced will continue to be planted with the currently available GE cotton (USDA-ERS, 2010c). U.S. cotton will continue to play a role in global cotton production, and will continue to be a supplier in the international market. How and where that cotton will be used will be subject to global market conditions.

Global economic benefits from the adoption of Bt cotton were nearly $1.4 billion for the U.S., China, India, Australia, Mexico, Argentina, and South Africa (Frisvold and Reeves, 2007). U.S. benefits of the use of Bt cotton were over $200 million (Frisvold and Reeves, 2007). The adoption of Bt cotton technology has brought millions of hectares of cotton land back into production in China and has revolutionized India's cotton industry (Meyer et al., 2007). Economic evidence shows that international trade and productivity is enhanced by the adoption of Bt cotton and that these products can be channeled into suitable export markets. Increased global production of Bt cotton adoption led to a 3% reduction in the world cotton price (Frisvold and Reeves, 2007). This research also showed that individual countries obtained greater economic gains by adopting Bt cotton technology than if they did not adopt it, and further, found non-adopting regions to lose cotton market share to the adopting regions (Frisvold and Reeves, 2007). These conditions are not expected to change if TwinLink™ Cotton remains a regulated article.

Preferred Alternative: Trade Economic Environment

BCS intends to submit dossiers to the proper regulatory authorities of foreign governments to allow the importation of U.S. cottonseed and to have regulatory processes in place so as to allow for the international movement of this product (Scott, 2008). These may include submissions to the relevant regulatory authorities in Canada, Mexico, the European Union (EU), and Japan, among others. TwinLink™ Cotton has been, or is currently, in field trials in cotton growing regions around the world (Scott, 2008).

A determination of nonregulated status of TwinLink™ Cotton is not expected to adversely impact the trade economic environment and may potentially enhance it through the subsequent development and global adoption of the TwinLink™ Cotton line which could provide another lepidopteran insect resistance and herbicide-tolerant management choice for growers. A reduction in costs for domestic growers associated with the reduction in insecticide and herbicide use may make U.S. producers more competitive in the global market. The trade economic impacts associated with the deregulation of TwinLink™ Cotton are anticipated to be very similar to the No Action Alternative.

Cumulative Effects: Trade Economic Environment

Current and historic economic evidence indicates that Bt cotton technology enhances international cotton trade and production. Based on the information described above, APHIS has determined that there are no past, present, or reasonable foreseeable actions that in aggregate with effects of the proposed action would negatively impact the trade economic environment.

4.7.3 Social Environment at Risk

U.S. cotton farms and their operators are similar in many respects to those of other crops, but are very different in some key areas. According to data from the 2003 Agricultural Resource Management Survey (ARMS), farms growing cotton tend to be larger than those growing other crops, with above average gross farm incomes, government payments, farm expenses, net incomes, farm asset values, and debt-to-asset ratios (Meyer et al., 2007).

Large farm operations are more likely to be organized into partnerships, and cotton farms are no exception. Partnerships allow operators to pool their resources to achieve economies of scale and to combine their talents in managing the farm operation (Meyer et al., 2007). Cotton farm operators are also more likely to list farming as their occupation and to have completed high school and college compared with other farm operators (Meyer et al., 2007).

Cotton farms in 2003 generated an average net cash income of $127,354 per farm, far more than the average of $11,568 for non-cotton farms in the cotton production regions (USDA-ERS, 2009). The higher average income generated on cotton farms is mainly due to their larger farm operations. Cotton farms averaged 1,199 acres per farm, compared with 376 acres for non-cotton farms (USDA-ERS, 2009). Cotton farms' average ratio of cash expenses to gross cash income was 71%, compared with 91% for non-cotton farms (USDA-ERS, 2009). This means that cotton farms could generate $100 of gross income with less expenditures (i.e., cotton farms can earn $100 of gross income for each $71 of cash expense compared to $91 of cash expense for a non-cotton farm). Larger farms can achieve economies of scale by spreading management, labor, and machinery costs over more units of output, thus gaining an advantage over smaller farms (USDA-ERS, 2009).

The household income for cotton producers averaged $142,463 in 2003. In comparison, the household income for non-cotton farms in cotton-producing states averaged $71,447 (USDA-ERS, 2009). For most farm households, income from off-farm sources exceeds income from the farm operation. However, cotton producers derive the majority of their family income from the farm. Eleven percent of cotton producers listed nonfarm jobs or businesses as their main occupation, compared with 48% of non-cotton farm operators (USDA-ERS, 2009).

No Action: Social Environment

Under the No Action Alternative, no direct impact on the social environment surrounding cotton farming is expected. The cropping and marketing decisions made by cotton growers are unlikely to be influenced by the selection of this alternative.

Preferred Alternative: Social Environment

Most of the cotton acreage in the U.S. is planted with GE cotton. Of the 11.3 million acres planted in cotton in 2010, 93% were GE cotton: 73% of that GE cotton acreage was GE insect-resistant (Bt) cotton and 78% was herbicide-tolerant (USDA-ERS, 2010a, 2010c). Most cotton is planted on farms that have been in cotton production for at least five years (USDA-NASS, 2007).

The introduced stacked lepidopteran-resistant and herbicide-tolerant trait in TwinLink™ Cotton is not intended to confer any competitive advantage in terms of weediness or to extend the range of cultivation outside of existing cultivation areas. A determination of nonregulated status of TwinLink™ Cotton by APHIS is not expected to significantly expand the number of cotton acres, and cotton acreage is expected to remain relatively stable. Overall impacts are similar to the No Action Alternative.

Cumulative Effects: Social Environment

Based on the information described above, APHIS has determined that there are no past, present, or reasonably foreseeable actions that in aggregate with effects of the proposed action would impact the social environment surrounding cotton farming.

4.8 OTHER CUMULATIVE EFFECTS

The potential cumulative effects regarding specific issues have been analyzed and addressed above. No further potential cumulative effects have been identified. To date, none of the GE cotton varieties that have been deregulated pursuant to Part 340 and the Plant Protection Act and used for commercial cotton production or cotton breeding programs have been subsequently found to pose a plant pest risk.

Cultivation of stacked varieties, those crop varieties that may contain more than one trait, are currently found in the marketplace and in agricultural production. In the event APHIS reaches a determination of nonregulated status, TwinLink™ Cotton may be combined with non-GE and GE cotton varieties by traditional breeding techniques, resulting in a plant that, for example, may also be resistant to other herbicides, or may present a different combination of insect pest-resistant, but may also have progeny with no transgenes at all. APHIS' current regulations at 7 CFR Part 340 do not provide for Agency oversight of GE cotton varieties previously deregulated pursuant to Part 340 and the Plant Protection Act, nor over stacked varieties combining deregulated GE varieties unless it can be positively shown that such stacked varieties were to pose a likely plant pest risk.

There is no guarantee that TwinLink™ Cotton will be stacked with any particular deregulated GE variety, as company plans and market demands play a significant role in those business decisions. Predicting all potential combinations of stacked varieties that could be created using both deregulated GE cotton varieties and also non-GE cotton varieties is hypothetical and purely speculative. No further analysis is required.

4.9 THREATENED AND ENDANGERED SPECIES

The Endangered Species Act (ESA) of 1973, as amended, is one of the most far-reaching wildlife conservation laws ever enacted by any nation. Congress, on behalf of the American people, passed the ESA to prevent extinctions facing many species of fish, wildlife and plants. The purpose of the ESA is to conserve endangered and threatened species and the ecosystems on which they depend as key components of America's heritage. To implement the ESA, the U.S. Fish and Wildlife Service (USFWS) works in cooperation with the National Marine Fisheries Service (NMFS), other Federal, State, and local agencies, Tribes, non-governmental organizations, and private citizens. Before a plant or animal species can receive the protection provided by the ESA, it must first be added to the Federal list of threatened and endangered wildlife and plants.

A species is added to the list when it is determined by the USFWS/NMFS to be endangered or threatened because of any of the following factors:

- The present or threatened destruction, modification, or curtailment of its habitat or range;

- Overutilization for commercial, recreational, scientific, or educational purposes;
- Disease or predation;
- The inadequacy of existing regulatory mechanisms; and
- The natural or manmade factors affecting its survival.

Once an animal or plant is added to the list, in accordance with the ESA, protective measures apply to the species and its habitat. These measures include protection from adverse effects of Federal activities.

Section 7 (a)(2) of the ESA requires that Federal agencies, in consultation with USFWS and/or the NMFS, ensure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of a listed species or result in the destruction or adverse modification of designated critical habitat. It is the responsibility of the Federal agency taking the action to assess the effects of their action and to consult with the USFWS and NMFS if it is determined that the action "may affect" listed species or critical habitat. To facilitate APHIS' ESA consultation process, APHIS met with the USFWS to discuss factors relevant to their effects analysis for petitions for nonregulated status and developed the following process for conducting an effects determination for biotechnology regulatory actions. Appendix A provides a copy of the interagency consultation protocol.

As part the environmental review process, APHIS thoroughly reviews GE product information and data to inform the ESA effects analysis and, if necessary, the biological assessment. For each transgene(s)/transgenic plant the following information, data, and questions are considered by APHIS:

- A review of the biology, taxonomy, and weediness potential of the crop plant and its sexually compatible relatives;
- Characterization of each transgene with respect to its structure and function and the nature of the organism from which it was obtained;
- A determination of where the new transgene and its products (if any) are produced in the plant and their quantity;
- A review of the agronomic performance of the plant including disease and pest susceptibilities, weediness potential, and agronomic and environmental impact;
- Determination of the concentrations of known plant toxicants (if any are known in the plant); and
- Analysis to determine if the transgenic plant is sexually compatible with any threatened or endangered plant species (TES) or a host of any TES.

In following this process, APHIS evaluated the potential effects that a determination of nonregulated status of TwinLink™ Cotton would have on Federally listed threatened and endangered species (TES) and species proposed for listing, as well as designated critical habitat and habitat proposed for designation. Based upon the scope of the EA and production areas identified in the Affected Environment section of the EA, APHIS obtained a list of TES species (listed and proposed) for each state where cotton is commercially produced from the USFWS Environmental Conservation Online System (ECOS; see Appendix B).

4.9.1 Potential Effects of TwinLink™ Cotton and Agricultural Practices on TES

BCS anticipates that TwinLink™ Cotton would replace currently available Bt cotton varieties and may be selected by growers of conventional cotton varieties making a change to a GE variety; however, availability of TwinLink™ Cotton is not expected to result in an increase in cotton acreage (Scott, 2008). Accordingly, the issues discussed herein focus on the potential environmental consequences of the deregulation of TwinLink™ Cotton on TES species in the areas where cotton is grown.

APHIS therefore focuses its TES review on the implications of exposure to the PAT and Cry proteins in cotton. This analysis also addresses the potential impacts of the use of glufosinate ammonium, and impacts to non-target organisms and the natural environment.

BCS has presented data evaluating the agronomic and morphological characteristics of TwinLink™ Cotton, including compositional and nutritional characteristics, safety evaluations and toxicity tests, comparing the product to a conventional cotton variety (i.e., Coker 315) (Scott, 2008). The results presented by BCS show no differences between TwinLink™ Cotton and the non-transgenic Coker 315 variety, other than the introduced PAT and Cry proteins. (See previous discussions on Human Health and Animal Feed for an expanded discussion of BCS' comparison.)

The agronomic and morphologic characteristics data provided by BCS were used in APHIS analysis of the weediness potential for TwinLink™ Cotton, and evaluated for the potential to impact TES. Agronomic studies conducted by BCS tested the hypothesis that the weediness potential of TwinLink™ Cotton is unchanged with respect to conventional cotton (Scott, 2008). No differences were detected between TwinLink™ Cotton and nontransgenic cotton in growth, reproduction, or interactions with pests and diseases, other than the intended effect of protection from lepidopteran pests. The main natural controls on feral populations of cotton are poor seed dispersal, poor seed germination, competition from other plants, and a high requirement for moisture (US-EPA, 2001b). Therefore, the incorporation of either the insect resistance trait or the herbicide tolerance trait is unlikely to appreciably improve seedling establishment and increase weediness potential. Any TwinLink™ Cotton volunteers could be readily controlled by other readily available herbicides, including glyphosate (Miller et al., 2004; Stewart et al., 2003). Based on this analysis, APHIS has concluded that deregulation of TwinLink™ Cotton has no potential to affect TES, as a result of weediness.

In addition to evaluating BCS' comparisons of TwinLink™ Cotton with the non-transgenic Coker 315 variety, for potential differences in agronomic and morphology APHIS also considers the EPA and FDA regulatory assessment in making its determination of the potential impacts of deregulation of the new agricultural product. In that regard, BCS has submitted food and feed safety and nutritional assessments to the FDA as well as comparable exemptions from tolerance and registration reviews to the EPA for TwinLink™ Cotton. The FDA and EPA are currently reviewing the information submitted by the applicant. In the absence of a completed FDA and EPA determinations, APHIS takes into consideration prior agency reviews of comparable products to conduct its assessment of the potential impacts. As discussed above in Animal and Plant Communities (Subsection 4.4) and Public Health (Subsection 4.5), both agencies have previously reviewed and approved comparable products, including several crop varieties expressing the Bt-based Cry proteins as plant-incorporated protectants, as well as the *bar* gene, resulting in the expression of the PAT enzyme providing tolerance to glufosinate ammonium.

Data regarding the environmental safety of PAT proteins have been extensively reviewed in international scientific, peer-reviewed journals (Hérouet et al., 2005). In summary, the PAT protein is expressed in a number of GM crops that have been approved by regulatory agencies. These GM crops have been consumed by humans and animals since 1995 and have exhibited no significant adverse effects. The PAT protein mode of action is specific to the breakdown of ammonia in plant tissues. Because animals lack this enzymatic pathway (Hérouet et al., 2005), and there is no mechanism to transfer this gene to unrelated plant species, APHIS limits its analysis of the implications of exposure to the PAT protein to the potential for gene movement to related TES cotton species.

None of the relatives of cotton are listed (or proposed) as endangered or threatened species under Federal (US-FWS, 2011a) or state listings (Arizona, 2009; Hawaii, 2001) with the exception of *G. hirsutum* in the State of Florida, where wild populations of upland cotton, *G. hirsutum* have been listed as endangered by the state (Coile and Garland, 2003; Institute for Regional Conservation, 2010). However, in Florida, wild *G. hirsutum* is not present in the northwestern panhandle where cotton cultivation occurs, and cultivation of cotton is prohibited by the EPA in those areas of southern Florida where it is found (Coile and Garland, 2003; US-EPA, 2008a; USDA-NASS, 2007; Wunderlin and Hansen, 2010). Accordingly, the deregulation of TwinLink™ Cotton is not expected to impact state endangered feral cotton populations.

Evaluation of the potential exposure of threatened and endangered species to the Bt-based Cry proteins focuses on the route of exposure. Cry1Ab and Cry2Ae proteins are highly specific to Lepidoptera. The specificity of Bt crystalline proteins to lepidopteran insect larvae, but not for other insects, birds, and mammals results from the highly specific receptors for these proteins in the larvae midgut (Lemaux, 2009). Once activated by insect-specific proteases in the insect midgut, Cry proteins bind to receptors in the midgut. Such binding leads to the formation of pores in the midgut membranes and ultimately to cell lysis and death. The specific binding of Bt-based Cry proteins to midgut membrane receptors is a key determinant of pest specificity (Showalter et al., 2009). Accordingly, the APHIS review of the potential impacts is focused on Federally listed threatened and endangered lepidopterans in the cotton growing regions of the U.S.

APHIS obtained and reviewed the USFWS Federal List of listed (or proposed) TES and considered the potential impacts of a determination of nonregulated status of TwinLink™ Cotton on lepidopterans (Appendix B). There are currently 21 species of butterflies, moths, and skippers on this list (US-FWS, 2011b). Because it is not possible to use TES species to quantify sensitivity to the Cry and PAT proteins, APHIS' evaluation focused on the likelihood of exposure of TES species to the Bt-endotoxin expressed in TwinLink™ Cotton. Exposure of TES species to the Bt-based Cry proteins in TwinLink™ Cotton is only likely if the species occur in the areas where cotton is grown, as cotton plant parts (seeds, pollen, and crop debris) are not readily transported long distances without human involvement.

APHIS has thoroughly examined all listed and proposed threatened and endangered lepidopterans and compared their habitats to those habitats present in counties where cotton is grown. APHIS has determined that the breeding habitats of listed Lepidoptera do not overlap cotton growing areas. Therefore, it is highly unlikely that these species can be exposed to TwinLink™ Cotton. Habitats in which threatened and endangered lepidopterans in the U.S. are likely to occur are very limited in geographical distribution. Moreover, the larvae of these species typically feed on specific host plants, none of which include cotton, or plants likely to be found in cotton fields. In those states where cotton is cultivated, lepidopteran species on the Federal TES list only occur in California, Florida, and North Carolina (US-FWS, 2011b) (see Appendix B: Threatened and Endangered Species). Of these species, only the Quino Checkerspot butterfly (*Euphydryas editha quino*), the Kern primrose sphinx moth (*Euproserpinus euterpe*), and the Saint Francis' satyr (*Neonympha mitchellii francisci*) are known to occur in counties were cotton is grown.

The Quino Checkerspot butterfly is found in coastal sage scrub, open chaparral, juniper woodland, and native grassland (US-FWS, 2003). Its primary host plants include: the dwarf plantain (*Plantago erecta*) that occurs in southern California within annual scrub, grassland, and open chaparral plant communities; the wooly plantain (*Plantago patagonica*) that overlaps in distribution with *P. erecta* at lower elevations; the white snapdragon (*Antirrhinum coulterianum*) that appears to be a facultative fire-follower in non-desert areas and in desert plant communities often growing between shrubs; and the thread-leaved bird's beak (*Cordylanthus rigidus*), a partially parasitic plant often found at high densities in disturbed areas, open slopes, and flats of foothill woodlands, chaparral margins, and coniferous forests (US-FWS, 2003). Areas where cotton is grown are likely to have been used in agricultural production for many years and thus would not change the habitat that is already unsuitable for the Quino Checkerspot butterfly.

The Kern primrose sphinx moth is also found in California. This moth may occupy habitat near cotton cultivation, known to occur in Kern, Santa Barbara, San Luis Obispo, and Ventura Counties, CA (Jump et al., 2006; US-FWS, 2007a). The larvae feed primarily on their essential host plant *Camissonia sp*. (certain primrose and sun cup species). The host plant evening primrose is found along sandy washes with young alluvial sandy soils that are prone to regular flooding (Jump et al., 2006). Since listing, the primary threats to the Kern primrose sphinx moth are agricultural land use practices that degrade the moth habitat, particularly cattle grazing, disking, pesticide and herbicide use, and development. Many agricultural pesticides are specifically designed to target insect larvae (caterpillars) as well as adult moths (US-FWS, 2007a). All Kern primrose sphinx moth populations are potentially at risk from this effect. Kern primrose sphinx moth exposure to agricultural pesticides could occur when cotton is grown in and around sandy washes with sandy alluvial soil where moths perform morning basking and where the food plant *Camissonia campestris* or *C. contorta* (field primrose and evening primrose, respectively) is supported. The potential route of Kern primrose sphinx moth exposure to the Bt protein contained in TwinLink™ Cotton would be in the form of pollen deposited on the evening primrose, where the threatened caterpillars feed. Because cotton pollen is heavy and will not be dispersed much from the field (Thies, 1953), it is not likely that the larvae of the Kern primrose sphinx moth will be exposed to the Cry protein. Any pollen produced by TwinLink™ Cotton plants is not expected to drift onto larval host plants.

The Saint Francis' satyr is known only in the sand hills of North Carolina, in Cumberland and Hoke Counties (US-FWS, 2010a). The species is known only to be located on government land (Fort Bragg U.S. Army installation). The habitat occupied by this satyr consists primarily of wide, wet meadows dominated by a high diversity of sedges (*Carex*) and other wetland graminoids. Saint Francis' satyr has also been observed in pitcher plant (*Sarracenia flava*) swales, with cane (*Arundinaria tecta*), rough-leaved loosestrife (*Lysimachia asperulaefolia*), and pocosin lily (*Lilium iridollai*). It is unknown whether satyr uses such habitat for reproduction or simply as a dispersal corridor. Adult food habits are not really known; larva probably feed on *Carex*, but this has not been established. Oviposition has been observed on small Panicum grass. Areas in Hoke and Cumberland County where cotton is grown are likely to have been used in agricultural production for many years and would be unsuitable habitat for the Saint Francis' satyr.

As noted above, the EPA conducts a comprehensive assessment of the implications of pesticide registrations on TES. EPA's evaluations include exposure assessments and ecological effects data for each registered pesticide. The EPA (2001b, 2008a) has published the following specific statements regarding potential effects of Bt-based Cry proteins incorporated in cotton—

> …The larvae of endangered Lepidoptera species in cotton growing counties (Quino Checkerspot butterfly, Saint Francis' satyr butterfly and Kern primrose sphinx moth) are not going to be exposed to Cry1Ac protein [the Bt-based Cry protein that was the focus of this report] because their habitats do not overlap with cotton fields. The Quino Checkerspot butterfly is found only in the coastal sage scrub habitat in southern California, the Kern primrose sphinx moth (threatened, not endangered) is found only on a privately owned ranch in the Walker Basin, Kern County California. Finally, the only known population of Saint Francis' satyr butterfly is found in wetlands dominated by sedges and grasses on Department of Defense property in North Carolina. None of the larvae of these insects feed on cotton, and will not be exposed to Cry protein in pollen. The amount of pollen that would drift from these cotton plants onto plants fed upon by endangered/threatened species, would be very small (if measurable) compared to the levels fed to the test species. Therefore, EPA does not expect that any endangered/threatened species will be affected by pollen containing the Cry1Ac protein.

In addition, because EPA is imposing conditions for geographic areas (Hawaii and Florida) that have sexually compatible wild or weedy relatives of cotton, the Cry1Ac protein gene cannot escape into related wild plants which could serve as a source of Bt pollen for plants on which endangered/threatened species may feed on in these areas.

Because EPA expects that no listed endangered species of Lepidoptera will be exposed to the Bt Cry protein expressed in cotton plants, and because the most probable exposure scenario does not appear to affect listed species, EPA believes that this action will have no effect on listed species (US-EPA, 2001b; see also US-EPA 2008a).

Based on the information above, APHIS has determined there would be no effects to the three listed lepidopteran species as a result of a determination of nonregulated status of TwinLink™ Cotton.

4.9.2 Potential Effects of the Use of Glufosinate Ammonium

As the action agency for pesticide registrations, EPA has the responsibility to conduct an assessment of effects of a registration action on endangered species. The EPA Endangered Species Protection Program web site, http://www.epa.gov/espp/, describes the EPA assessment process for endangered species. Some of the elements of that process, generally taken from the web site, are summarized below.

When registering a pesticide or reassessing the potential ecological risks from use of a currently registered pesticide, EPA evaluates extensive exposure and ecological effects data to determine how a pesticide will move through and break down in the environment. Risks to birds, fish, invertebrates, mammals, and plants are routinely assessed and used in EPA's determinations of whether a pesticide may be licensed for use in the U.S.

EPA's core pesticide risk assessment and regulatory processes ensure that protections are in place for all populations of nontarget species, including TES. These assessments provide EPA with information needed to develop label use restrictions for the pesticide. These label restrictions carry the weight of law and are enforced by EPA and the states (Federal Insecticide, Fungicide, and Rodenticide Act 7 USC 136j (a)(2)(G) Unlawful acts). Because TES may need specific protection, EPA has developed risk assessment procedures described in the Overview of the Ecological Risk Assessment Process (US-EPA, 2004) to determine whether individuals of a listed species have the potential to be harmed by a pesticide, and if so, what specific protections may be appropriate. EPA's conclusion regarding the potential risks a pesticide may pose to a listed species and any designated critical habitat for the species, after conducting a thorough ecological risk assessment, results in an "effects determination" in accordance with Section 7 (a)(2) of the ESA.

The EPA first registered glufosinate ammonium in 2000 as a non-selective foliar herbicide for use on a wide range of crops, including cotton (US-EPA, 2008b). In 2008, the EPA announced that it was undertaking a registration review of this product, and has published a preliminary work plan for this process (US-EPA, 2008b). The EPS' preliminary work plan provides for an ecological risk assessment (ERA) to allow the EPA to determine whether glufosinate ammonium use has "no effect" or "may affect" Federally listed threatened or endangered species (listed species) or their designated critical habitat (US-EPA, 2008b). If the ERA indicates that glufosinate ammonium "may affect" a listed species or its designated critical habitat, the assessment will be refined to allow the EPA to determine whether the use of glufosinate ammonium is "likely to adversely affect" the species or critical habitat or "not likely to adversely affect" the species or critical habitat (US-EPA, 2008b). When an ERA concludes that a pesticide's use "may affect" a listed species or its designated critical habitat, the Agency will consult with the U.S. Fish and Wildlife Service and National Marine Fisheries Service, as appropriate.

To mitigate potential adverse effects to TES, EPA has imposed specific label use restrictions for glufosinate ammonium use when applied with aerial equipment including “The product should only be applied when the potential for drift to adjacent sensitive areas (e.g., residential areas, bodies of water, known habitat for threatened or endangered species, non-target crops) is minimal (e.g., when wind is blowing away from the sensitive areas)” (BCS, 2010a).

To facilitate pesticide applicators adherence to EPA label use restrictions for glufosinate ammonium, BCS’ label for Ignite, the commercial form of glufosinate ammonium, provides specific instructions for managing spray drift and cautions pertaining to applications around water so as to minimize potential effects to non-target organisms (BCS, 2010a). Label requirements for BCS’ Ignite formulations prohibit application in conditions or locations where adverse impact on Federally listed endangered/threatened plants or aquatic species is likely (BCS, 2010a). BCS has also provided a technical and trade manual with additional guidance on the proper storage, handling and use of the product (BCS, 2010b).

In conclusion, there are legal precautions in place (EPA label use restrictions) and “best practice” guidance to reduce the possibility of exposure and adverse impacts to TES from glufosinate ammonium application to TwinLink™ Cotton; EPA has considered potential impacts to TES as part of their registration and labeling process for glufosinate ammonium; and adherence to EPA label use restrictions by the pesticide applicator will ensure that the use of glufosinate ammonium will not adversely affect TES or critical habitat. Based on these factors and the legal requirements for pesticide applicators to follow EPA label use restrictions, APHIS has determined that the use of EPA registered glufosinate ammonium for TwinLink™ Cotton production will not adversely impact listed species or species proposed for listing and would not adversely impact designated critical habitat or habitat proposed for designation.

Based on the above information, APHIS has determined that the Preferred Alternative, a determination of nonregulated status of TwinLink™ Cotton, would have no effect on Federally listed TES and species proposed for listing, or on designated critical habitat or habitat proposed for designation. Consequently, a written concurrence or formal consultation with the USFWS is not required for this action.

4.10 CONSIDERATION OF EXECUTIVE ORDERS, STANDARDS, AND TREATIES RELATING TO ENVIRONMENTAL IMPACTS

4.10.1 Executive Orders with Domestic Implications

The following two executive orders require consideration of the potential impacts to minority and low income populations and children:

- ***Executive Order (EO) 12898 (US-NARA, 2010), "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations,"*** requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.

- ***EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks,"*** acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children.

Each alternative was analyzed with respect to EO 12898 and EO 13045. Neither alternative is expected to have a disproportionate adverse effect on minorities, low-income populations, or children.

Available mammalian toxicity, along with the history of safe use of microbial Bt products and other cotton varieties expressing Bt and PAT proteins, establishes the safety of TwinLink™ Cotton and its products to humans, including minorities, low income populations, and children who might be exposed to them through agricultural production and/or processing. No additional safety precautions would need to be taken.

None of the impacts on agricultural practices expected to be associated with deregulation of TwinLink™ Cotton are expected to have a disproportionate adverse effect on minorities, low income populations, or children. As noted above, the cultivation of previously deregulated cotton varieties with similar insect-resistant traits has been associated with a decrease and/or shift in pesticide applications for those who adopt these varieties that is either favorable or neutral with respect to environmental and human toxicity. If pesticide applications are reduced, there may be a beneficial effect on children and low income populations that might be exposed to the chemicals. These populations might include migrant farm workers and their families, and other rural dwelling individuals who are exposed to pesticides through groundwater contamination or other means of exposure. It is expected that EPA and USDA Economic Research Service would monitor the use of this product to determine impacts on agricultural practices, such as chemical use, as they have done previously for other products.

The following executive order addresses Federal responsibilities regarding the introduction and effects of invasive species:

> ***EO 1311 (US-NARA, 2010), "Invasive Species,"*** states that Federal agencies take action to prevent the introduction of invasive species, to provide for their control, and to minimize the economic, ecological, and human health impacts that invasive species cause.

Non-engineered cotton as well as other Bt and herbicide-tolerant cotton varieties are widely grown in the U.S. Based on historical experience with these varieties and the data submitted by the applicant and reviewed by APHIS, TwinLink™ Cotton plants are sufficiently similar in fitness characteristics to other cotton varieties currently grown and are not expected to become weedy or invasive (USDA-APHIS, 2010b).

The following executive order requires the protection of migratory bird populations:

> ***EO 13186 (US-NARA, 2010), "Responsibilities of Federal Agencies to Protect Migratory Birds,"*** states that Federal agencies taking actions that have, or are likely to have, a measurable negative effect on migratory bird populations are directed to develop and implement, within two years, a Memorandum of Understanding (MOU) with the Fish and Wildlife Service that shall promote the conservation of migratory bird populations.

Data submitted by the applicant has shown no difference in compositional and nutritional quality of TwinLink™ Cotton compared with other GE cotton or non-GE cotton, apart from the presence of the two Cry proteins and the PAT protein. The migratory birds that occasionally forage in cotton fields are unlikely to ingest high amounts of TwinLink™ Cotton seed as cotton seed is limited by harvest.

The introduction of Bt cotton has had a positive effect on bird counts in North American cotton fields. Comparing bird populations from 1991 to 1995 and 1996 to 2000, EPA (2001b) showed that bird counts increased and were positively correlated with Bt cotton adoption, the reduction in insecticide use, and the relative presence of the species in cotton fields prior to the introduction of Bt cotton (US-EPA, 2001b). Broad-spectrum insecticides, which are considered to be one of the largest impediments to agroecosystem biodiversity, have been reduced by the commercial use of Bt cotton (Benbrook, 2009; USDA-NASS, 2008). Since the introduction of Bt cotton there has been a two thirds decrease in use for the most toxic insecticides products to birds and fish, and a comparable one third decrease in use for the most toxic products to humans (US-EPA, 2001b). Additionally, there have been no reported adverse effects of Bt cotton on non-target insects which can serve as a food source for birds.

Collectively, the weight of considerable field studies with Bt crops, including Bt cotton, have consistently demonstrated that Bt crops have only minor effects, if any, on a large number of non-target insect taxa which is very small in comparison to the use of broad-spectrum insecticides (Naranjo, 2009a).

The history of safe use of crops expressing the PAT enzyme strongly suggests that TwinLink™ Cotton will not present a risk to birds. APHIS deregulated the PAT enzyme *bar* gene construct in cotton in 2003 with the BCS LibertyLink® Cotton (USDA-APHIS, 2003). In 2003, the FDA completed its food safety consultation on LibertyLink® Cotton, and granted tolerance exemptions for both the plant-incorporated protectant as well as the genetic material necessary for its production (US-FDA, 2003). The EPA granted exemptions from the requirement of a tolerance for residues of glufosinate ammonium as well as the plant-pesticide PAT in or on food and feed commodities of cotton (see: 62 FR 17719; 40 CFR §180.473; US-EPA, 2010c). When used in accordance with label restrictions, glufosinate ammonium does not present a risk to migratory birds.

Based on APHIS' assessment of TwinLink™ Cotton, it is unlikely that a determination of nonregulated status of this cotton variety will have a negative effect on migratory bird populations.

4.10.2 International Implications

EO 12114 (US-NARA, 2010), "Environmental Effects Abroad of Major Federal Actions" requires Federal officials to take into consideration any potential environmental effects outside the U.S., its territories, and possessions that result from actions being taken.

APHIS has given this EO carefulconsideration and does not expect a significant environmental impact outside the U.S. in the event of a determination of nonregulated status of TwinLink™ Cotton.

It should be noted that all the existing national and international regulatory authorities, and phytosanitary regimes that currently apply to introductions of new cotton cultivars internationally apply equally to those covered by an APHIS determination of nonregulated status under 7 CFR Part 340.

Any international trade of TwinLink™ Cotton subsequent to a determination of nonregulated status of the product would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC, 2010). The purpose of the IPPC "is to secure a common and effective action to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control" (IPPC, 2010). The protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds.

The IPPC establishes a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (172 countries as of March 2010). In April 2004, a standard for PRA of living modified organisms (LMOs) was adopted at a meeting of the governing body of the IPPC as a supplement to an existing standard, International Standard for Phytosanitary Measure No. 11 (ISPM-11, Pest Risk Analysis for Quarantine Pests). The standard acknowledges that all LMOs will not present a pest risk and that a determination needs to be made early in the PRA for importation as to whether the LMO poses a potential pest risk resulting from the genetic modification. APHIS pest risk assessment procedures for genetically engineered organisms are consistent with the guidance developed under the IPPC. In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in other international forums and through national regulations.

The ***Cartagena Protocol on Biosafety*** is a treaty under the United Nations Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of LMOs, which include those modified through biotechnology. The Protocol came into force on September 11, 2003, and 160 countries are Parties to it as of December 2010 (CBD, 2010). Although the U.S. is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, U.S.

exporters will still need to comply with those regulations that importing countries which are Parties to the Protocol have promulgated to comply with their obligations. The first intentional transboundary movement of LMOs intended for environmental release (field trials or commercial planting) will require consent from the importing country under an advanced informed agreement (AIA) provision, which includes a requirement for a risk assessment consistent with Annex III of the Protocol and the required documentation.

LMOs imported for food, feed, or processing (FFP) are exempt from the AIA procedure, and are covered under Article 11 and Annex II of the Protocol. Under Article 11, Parties must post decisions to the Biosafety Clearinghouse database on domestic use of LMOs for FFP that may be subject to transboundary movement. To facilitate compliance with obligations to this protocol, the U.S. Government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products (see the NBII listings posted at http://usbiotechreg.nbii.gov/). These data will be available to the Biosafety Clearinghouse.

APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines, and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the U.S., and within the Organization for Economic Cooperation and Development (OECD). NAPPO has completed three modules of the Regional Standards for Phytosanitary Measures (RSPM) No. 14, *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (NAPPO, 2003).

APHIS also participates in the ***North American Biotechnology Initiative (NABI)***, a forum for information exchange and cooperation on agricultural biotechnology issues for the U.S., Mexico, and Canada. In addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including Argentina, Brazil, Japan, China, and Korea.

4.10.3 Compliance with Clean Water Act and Clean Air Act

This EA evaluated the changes in cotton production due to the unrestricted use of TwinLink™ Cotton. Cultivation of TwinLink™ Cotton is not expected to lead to the increased production of cotton in U.S. agriculture.

There is no expected change in water use and quality due to the cultivation of TwinLink™ Cotton compared with current cotton seed and cotton production. Also, there is no expected change in air quality associated with the cultivation of TwinLink™ Cotton.

Based on this review, APHIS concludes that the cultivation of TwinLink™ Cotton would comply with the Clean Water Act and the Clean Air Act.

4.10.4 Impacts on Unique Characteristics of Geographic Areas

A determination of non-regulated status of TwinLink™ Cotton is not expected to impact unique characteristics of geographic areas such as park lands, prime farm lands, wetlands, wild and scenic areas, or ecologically critical areas.

The common agricultural practices that would be carried out in the cultivation of TwinLink™ Cotton are not expected to deviate from current practices. The product is expected to be deployed on agricultural land currently suitable for production of cotton and replace existing varieties, and is not expected to increase the acreage of cotton production.

There are no proposed major ground disturbances; no new physical destruction or damage to property; no alterations of property, wildlife habitat, or landscapes; and no prescribed sale, lease, or transfer of ownership of any property. This action is limited to a determination of non-regulated status of TwinLink™ Cotton. This action would not convert land use to nonagricultural use and therefore would have no adverse impact on prime farm land. Standard agricultural practices for land preparation, planting, irrigation, and harvesting of plants would be used on agricultural lands planted to TwinLink™ Cotton, including the use of EPA registered pesticides. The Applicant's adherence to EPA label use restrictions for all pesticides is expected to mitigate potential impacts to the human environment.

4.10.5 National Historic Preservation Act (NHPA) of 1966 as Amended

The NHPA of 1966 and its implementing regulations (36 CFR 800) require Federal agencies to: 1) determine whether activities they propose constitute "undertakings" that have the potential to cause effects on historic properties and 2) if so, to evaluate the effects of such undertakings on such historic resources and consult with the Advisory Council on Historic Preservation (i.e., State Historic Preservation Office, Tribal Historic Preservation Officers), as appropriate.

APHIS' proposed action, a determination of nonregulated status of TwinLink™ Cotton, is not expected to adversely impact cultural resources on tribal properties. Any farming activity that may be taken by farmers on tribal lands would only be conducted at the tribe's request; thus, the tribes would have control over any potential conflict with cultural resources on tribal properties.

APHIS' proposed action would have no impact on districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places, nor would it likely cause any loss or destruction of significant scientific, cultural, or historical resources.

APHIS' proposed action is not an undertaking that may directly or indirectly cause alteration in the character or use of historic properties protected under the NHPA. In general, common agricultural activities conducted under this action do not have the potential to introduce visual, atmospheric, or noise elements to areas in which they are used that could result in effects on the character or use of historic properties. For example, there is potential for increased noise on the use and enjoyment of a historic property during the operation of tractors and other mechanical equipment close to such sites. Nevertheless, it is expected that this noise would only be temporary and short-term. The cultivation of TwinLink™ Cotton is not expected to change any of these agronomic practices that would result in an adverse impact under the NHPA.

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APPENDIX A

APHIS THREATENED AND ENDANGERED SPECIES DECISION TREE FOR FWS CONSULTATIONS

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APHIS THREATENED AND ENDANGERED SPECIES DECISION TREE FOR FWS CONSULTATIONS

DECISION TREE ON WHETHER SECTION 7 CONSULTATION WITH FWS IS TRIGGERED FOR PETITIONS OF TRANSGENIC PLANTS

This decision tree document is based on the phenotypes (traits) that have been permitted for environmental releases under APHIS oversight (for a list of approved notifications and environmental releases, visit Information Systems for Biotechnology, at http://isb.vt.edu.) APHIS will re-evaluate and update this decision document as it receives new applications for environmental releases of new traits that are genetically engineered into plants.

BACKGROUND

For each transgene(s)/transgenic plant the following information, data, and questions will be addressed by APHIS, and the EAs on each petition will be publicly available. APHIS review will encompass:

- A review of the biology, taxonomy, and weediness potential of the crop plant and its sexually compatible relatives;
- Characterization of each transgene with respect to its structure and function and the nature of the organism from which it was obtained;
- A determination of where the new transgene and its products (if any) are produced in the plant and their quantity;
- A review of the agronomic performance of the plant including disease and pest susceptibilities, weediness potential, and agronomic and environmental impact;
- Determination of the concentrations of known plant toxicants (if any are known in the plant),
- Analysis to determine if the transgenic plant is sexually compatible with any threatened or endangered plant species (TES) or a host of any TES.

FDA published a policy in 1992 on foods derived from new plant varieties, including those derived from transgenic plants (http://vm.cfsan.fda.gov/~lrd/fr92529b.html and http://vm.cfsan.fda.gov/~lrd/consulpr.html). The FDA's policy requires that genetically engineered foods meet the same rigorous safety standards as is required of all other foods. Many of the food crops currently being developed using biotechnology do not contain substances that are significantly different from those already consumed by human and thus do not require pre-market approval. Consistent with its 1992 policy, FDA expects developers to consult with the agency on safety and regulatory questions. A list of consultations is available at http://vm.cfsan.fda.gov/~lrd/biocon.html. APHIS considers the status and conclusion of the FDA consultations in its EAs.

Below is a description of our review process to whether a consultation with U.S. Fish and Wildlife Service is necessary.

If the answer to any of the questions 1-4 below is yes, APHIS will contact FWS to determine if a consultation is required:

Is the transgenic plant sexually compatible with a TE plant[1] without human intervention?

1. Are naturally occurring plant toxins (toxicants) or allelochemicals increased over the normal concentration range in parental plant species?
2. Does the transgene product or its metabolites have any significant similarities to known toxins[2]?
3. Will the new phenotype(s) imparted to the transgenic plant allow the plant to be grown or employed in new habitats (e.g., outside agro-ecosystem)[3].
4. Does the pest resistance[4] gene act by one of the mechanisms listed below? If the answer is YES then a consultation with U.S. Fish and Wildlife Service is NOT necessary.

A. The transgene acts only in one or more of the following ways:

i. As a structural barrier to either the attachment of the pest to the host, to penetration of the host by the pest, to the spread of the pest in the host plant (e.g., the production of lignin, callose, thickened cuticles);
ii. In the plant by inactivating or resisting toxins or other disease causing substances produced by the pest;
iii. By creating a deficiency in the host of a component required for growth of the pest (such as with fungi and bacteria);
iv. By initiating, enhancing, or potentiating the endogenous host hypersensitive disease resistance response found in the plant;
v. In an indirect manner that does not result in killing or interfering with normal growth, development, or behavior of the pest;

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[1] APHIS will provide FWS a draft EA that will address the impacts, if any, of gene movement to the TES plant

[2] Via a comparison of the amino acid sequence of the transgene's protein with those found in the protein databases like PIR, Swiss-Prot and HIV amino acid data bases.

[3] Such phenotypes might include tolerance to environmental stresses such as drought, salt, frost, aluminum or heavy metals.

[4] Pest resistance would include any toxin or allelochemical that prevents, destroys, repels or mitigates a pest or effects any vertebrate or invertebrate animal, plant, or microorganism.

B. A pest derived transgene is expressed in the plant to confer resistance to that pest (such as with coat protein, replicase, and pathogen virulence genes).

For the biotechnologist:

Depending on the outcome of the decision tree, initial the appropriate decision below and incorporate its language into the EA. Retain a hard copy of this decision document in the petition's file.

________ BRS has reviewed the data in accordance with a process mutually agreed upon with the U.S. Fish and Wildlife Service to determine when a consultation, as required under Section 7 of the Endangered Species Act, is needed. APHIS has reached a determination that the release following a determination of non-regulated status would have no effects on listed threatened or endangered species and consequently, a written concurrence or formal consultation with the Fish and Wildlife Service is not required for this EA.

________ BRS has reviewed the data in accordance with a process mutually agreed upon with the U.S. Fish and Wildlife Service to determine when a consultation, as required under Section 7 of the Endangered Species Act, is needed. APHIS reached a determination that the release following a determination of non-regulated status is not likely to adversely affect any listed threatened or endangered species and consequently obtained written concurrence from the Fish and Wildlife Service.

________ BRS has reviewed the data in accordance with a process mutually agreed upon with the U.S. Fish and Wildlife Service to determine when a consultation, as required under Section 7 of the Endangered Species Act, is needed. APHIS reached a determination that the release following a determination of non-regulated status is likely to affect adversely one or more listed threatened or endangered species and has initiated a formal consultation with the Fish and Wildlife Service.

APPENDIX B

THREATENED AND ENDANGERED SPECIES STATE LISTS

APPENDIX B

THREATENED AND ENDANGERED SPECIES

Distribution, Habitat, and Food Sources of Threatened and Endangered Lepidoptera (US-FWS, 2011b)

Given the specificity of activity of the Cry proteins, species outside the insect order Lepidoptera should not be affected (Naranjo, 2009a; Sanvido et al., 2006). APHIS has thoroughly examined all FWS threatened and endangered lepidopteran species to determine if there could be possible effects from the proteins found in TwinLink™ Cotton. Threatened and endangered lepidopterans in the U.S. have restrictive habitats; and their larvae typically feed on specific host plants, none of which includes cotton or its sexually compatible relatives, or plants expected to be found in cultivated agricultural land. Furthermore, an examination of county distribution of endangered lepidopterans shows that most are not known to occur in counties where cotton is grown, with the exception of the Kern primrose sphinx moth, the Quino Checkerspot butterfly and the Saint Francis' satyr butterfly. The EPA (2001b) has already concluded that the habitats of these species do not overlap with cotton fields and their larvae do not feed on cotton. Pollen dispersal due to wind is considered insignificant because cotton pollen is sticky and heavy (Khan and Afzal, 1950; Thies, 1953). The EPA (2001b) has concluded that the amounts of cotton pollen that might be deposited on host plants of these species would be negligible, or perhaps none at all, and have no impact. If pollen were to deposit on host plants, the amount of pollen that would drift from these cotton plants onto plants fed upon by endangered/threatened species, would be very small compared to the levels fed to test species (Scott, 2008; US-EPA, 2008; USDA-APHIS, 2010b).

Species	Description
Butterfly, bay checkerspot *Euphydryas editha bayensis*	The Bay checkerspot butterfly (*Euphydryas editha bayensis*) is restricted to serpentine outcrops near San Francisco Bay, in Santa Clara and San Matao, CA. The primary constituent elements of the habitat for the bay checkerspot are one or more of the following: stands of *Plantago erecta*, *Castilleja exserta*, or *Castilleja densiflora*; spring flowers providing nectar; pollinators of the bay checkerspot's food and nectar plants; soils derived from serpentinic rock; and space for dispersal between habitable areas (NatureServe, 2010a; US-FWS, 2001a).
Butterfly, Behren's silverspot *Speyeria zerene behrensii*	Currently inhabits one site in southern Mendocino County, CA. The Behren's silverspot butterfly inhabits coastal prairie habitat that contains *Viola adunca* (early blue violet), the larval host plant, adult nectar sources, and adult courtship (NatureServe, 2010b; US-FWS, 2004).
Butterfly, callippe silverspot *Speyeria callippe callippe*	Is found in native grassland and associated habitats in the San Francisco Bay area, CA. The females lay their eggs on the dry remains of the larvae foodplant, Johnny jump-up (*Viola pedunculata*), or on the surrounding debris (Butterfly Conservation Initiative, 2006c; US-FWS, 2010b).

Species	Description
Butterfly, El Segundo blue *Euphilotes battoides allyni*	Now limited to one 302-acre parcel (owned by the Los Angeles Airport) and one 2-acre parcel (owned by the Standard Oil Company of California) in El Segundo Dunes, Los Angeles County, CA in sand dunes with its larval and adult host plant *Eriogonum parvifolium* (NatureServe, 2010c; US-FWS, 2007b).
Butterfly, Fender's blue *Icaricia icarioides fenderi*	Restricted primarily to the Willamette Valley of Oregon, in upland prairies in Douglas, Benton, Lane, Linn, Polk, and Yamhill Counties, OR and Lewis County, WA. Dry, fescue prairies make up the majority of habitat. Larvae feed exclusively on certain lupine, mainly *Lupinus sulphereus* var. *kincaid* occasionally *L. laxiflorus* and *albicauliss* (NatureServe, 2010d; US-FWS, 2000a).
Butterfly, Karner blue *Lycaeides melissa samuelis*	Requires the wild lupine (*Lupinus perennis*) that occurs in Illinois, Indiana, Michigan, Minnesota, New Hampshire, New York, Ohio, and Wisconsin to deposit eggs for larval food. In eastern New York and New Hampshire, habitat typically is in sandplain communities, such as grassy openings within very dry, sandy pitch pine/scrub oak barrens. In the Midwest, the habitat is also dry and sandy, including oak savanna and jack pine barrens, and less often dune communities. Within the overall community remnant inhabited by a metapopulation, any patch of foodplant in open to semi-shaded setting is likely to be used. Females lay eggs on or near wild lupine plants, and main requirement seems to be thousands of stems of lupine in the short term (NatureServe, 2010e).
Butterfly, Lange's metalmark *Apodemia mormo langei*	Is currently found only at Antioch Sand Dunes in Contra Costa County, CA, as part of the Antioch Dunes National Wildlife Refuge. The larvae are known to feed only on buckwheat (*Eriogonum nudum* ssp. *auriculatum*) (US-FWS, 2008).
Butterfly, lotis blue *Lycaeides argyrognomon lotis*	Is found only in Mendocino County, CA in a sphagnum-willow bog of about five acres in size. Larvae probably feed on *Lotus formosissimus*; if not then presumably some other legume such as *Lathyrus vestitus bolanderi*. If not a legume, some species of Ericaceae would seem most likely. (NatureServe, 2010f).
Butterfly, mission blue *Icaricia icarioides missionensis*	Restricted to a few sites in about three populations, including San Bruno Mountain in San Mateo County, Twin Peaks in San Francisco, and the vicinity of Skyline College in San Mateo County, CA. Limited to grasslands, with its larval hosts, *Lupinus albifrons*, *L. varicolor*, and *L. formosus* (NatureServe, 2010g).
Butterfly, Mitchell's satyr *Neonympha mitchellii mitchellii*	Known to occur in Indiana, Michigan, and Ohio in northern limestone wetlands. Females oviposit on a variety of small forbs and sedges (e.g., *Thelypeteris palustris*, and *Carex* sp.) seedlings and individuals do not move great distances (Barton and Bach, 2005; Szymanski et al., 2004; US-FWS, 1998a, 2010c).

Species	Description
Butterfly, Myrtle's silverspot *Speyeria zerene myrtleae*	Marin, San Mateo, and Sonoma, CA. Myrtle's silverspot inhabits coastal dunes, coastal prairie, and coastal scrub at elevations ranging from sea level to 300 meters (1,000 feet), and as far as 5 kilometers (3 miles) inland. The larval food plant is *Viola adunca* and possibly other *Viola* species (US-FWS, 1998b).
Butterfly, Oregon silverspot *Speyeria zerene hippolyta*	Known to occur in Del Norte County, CA; in Clatsop, Lane, Lincoln, Tillamook and Counties, OR and Pacific County, WA in salt-spray meadows with its host *Viola adunca* (US-FWS, 2001b).
Butterfly, Palos Verdes blue *Glaucopsyche lygdamus palosverdesensis*	Occurs only in Los Angeles County, CA, confined to coastal sage scrub community and is dependent on two known host plants, locoweed (*Astragalus trichopodus* var. *lonchus*, also known as Santa Barbara milkvetch) and common deerweed (*Lotus scoparius*) (Butterfly Conservation Initiative, 2006a; US-FWS, 1980).
Butterfly, Quino checkerspot *Euphydryas editha quino (=E. e. wrighti)*	Is known to occur in the San Diego National Wildlife Refuge, in Riverside and San Diego Counties, CA, in Chaparral, coastal sage scrub, with primary host plants: dwarf plantain (*Plantago erecta*), wooly plantain (*Plantago patagonica*), the white snapdragon (*Antirrhinum coulterianum*), thread-leaved bird's beak (*Cordylanthus rigidus*) (US-FWS, 2003, 2010d).
Butterfly, Saint Francis' satyr *Neonympha mitchellii francisci*	Located in one site in NC sandhills, may be restricted to artillery impact areas at Fort Bragg, military installation in North Carolina. Known only from a few sedge wetlands (NatureServe, 2010h).
Butterfly, San Bruno elfin *Callophrys mossii bayensis*	Restricted to Contra Costa, Marin and San Mateo Counties, CA. The San Bruno elfin is found in coastal mountains near San Francisco Bay, in the fog-belt of steep north facing slopes that receive little direct sunlight. It lives near prolific growths of the larval food plant, stonecrop (*Sedum spathulifolium*), which is a low-growing succulent. Stonecrop is associated with rocky outcrops that occur at 900-1075 feet elevation (Butterfly Conservation Initiative, 2006b; NatureServe, 2010i; US-FWS, 2010e).
Butterfly, Schaus swallowtail *Heraclides aristodemus ponceanus*	Restricted to Dade and Monroe Counties, FL. Habitat is tropical hardwood hammocks and their edges with the larval foodplant which is torchwood *Amyris elemifera*. Adults do stray into other nearby areas. Larvae feed mainly on *Amyris elemifera* and occasionally on other Rutaceae (NatureServe, 2010j; US-FWS, 1999).
Butterfly, Smith's blue *Euphilotes enoptes smithi*	Restricted to Monterey, Santa Cruz, and San Mateo counties, CA. Coastal and inland sand dunes and steep slopes along the coast where coastal sand dune strand vegetation dominates. One population found in chaparral-woodland dominated area. Also has been found in serpentine grassland area. Area must contain seacliff and coastal buckwheat. An undescribed ecotype of *E. latifolium* (coastal buckwheat) is used by the females for oviposition as well as providing food for the larvae (NatureServe, 2010k).

Species	Description
Butterfly, Uncompahgre fritillary *Boloria acrocnema*	Restricted to: Gunnison, Hinsdale, and Chaffee counties in CO; isolated alpine habitats in the San Juan Mountains of southwestern Colorado. Habitat is moist alpine slopes above 12,000 feet with extensive snow willow (*Salix nivalis)* (NatureServe, 2010l; US-FWS, 1994).
Moth, Blackburn's sphinx *Manduca blackburni*	, Maui and is more or less restricted to tracts of dry forest. Inhabits mostly lowland dry forests and shrub lands where the larvae feed on native and a few introduced Solanaceae (tobacco family). However, adults do wander and larvae have turned up on cultivated Solanaceae. Larvae of Blackburn's sphinx moth feed on plants in the nightshade family (Solanaceae). The natural host plants are native shrubs in the genus Solanum (popolo), and the native tree, *Nothocestrum latifolium* (`aiea), on which the larvae consume leaves, stems, flowers, and buds. However, many of the host plants recorded for this species are not native to the Hawaiian Islands, and include *Nicotiana tabacum* (commercial tobacco), Nicotiana glauca (tree tobacco), *Solanum melongena* (eggplant), *Lycopersicon esculentum* (tomato), and possibly *Datura stramonium* (Jimson weed) (NatureServe, 2010m; US-FWS, 2000b, 2010f).
Moth, Kern primrose sphinx *Euproserpinus euterpe*	Found in Kern, San Luis Obispo, Santa Barbara, and Ventura Counties, CA. The most important habitat factor is presence of the larval foodplant, which is *Camissonia* sp. Some of the habitat has been disked, and some roads and development are within the population areas. Sheep grazing has contributed to habitat destruction (US-FWS, 2007a).

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